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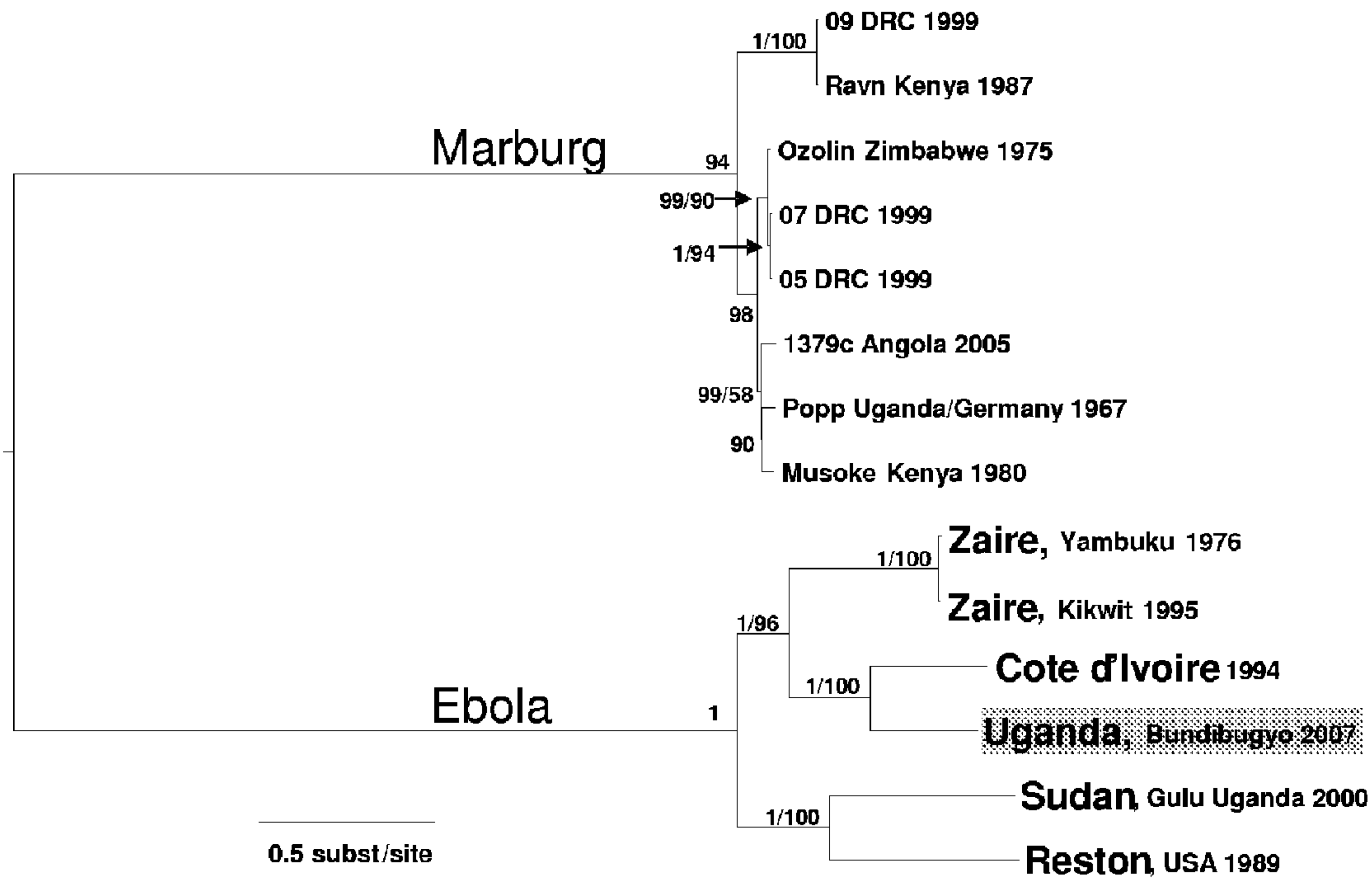


Fig. 1

(57) **Abrégé/Abstract:**

Compositions and methods including and related to the Ebola Bundibugyo virus (EboBun) are provided. Compositions are provided that are operable as immunogens to elicit and immune response or protection from EboBun challenge in a subject such as a primate. Inventive methods are directed to detection and treatment of EboBun infection.

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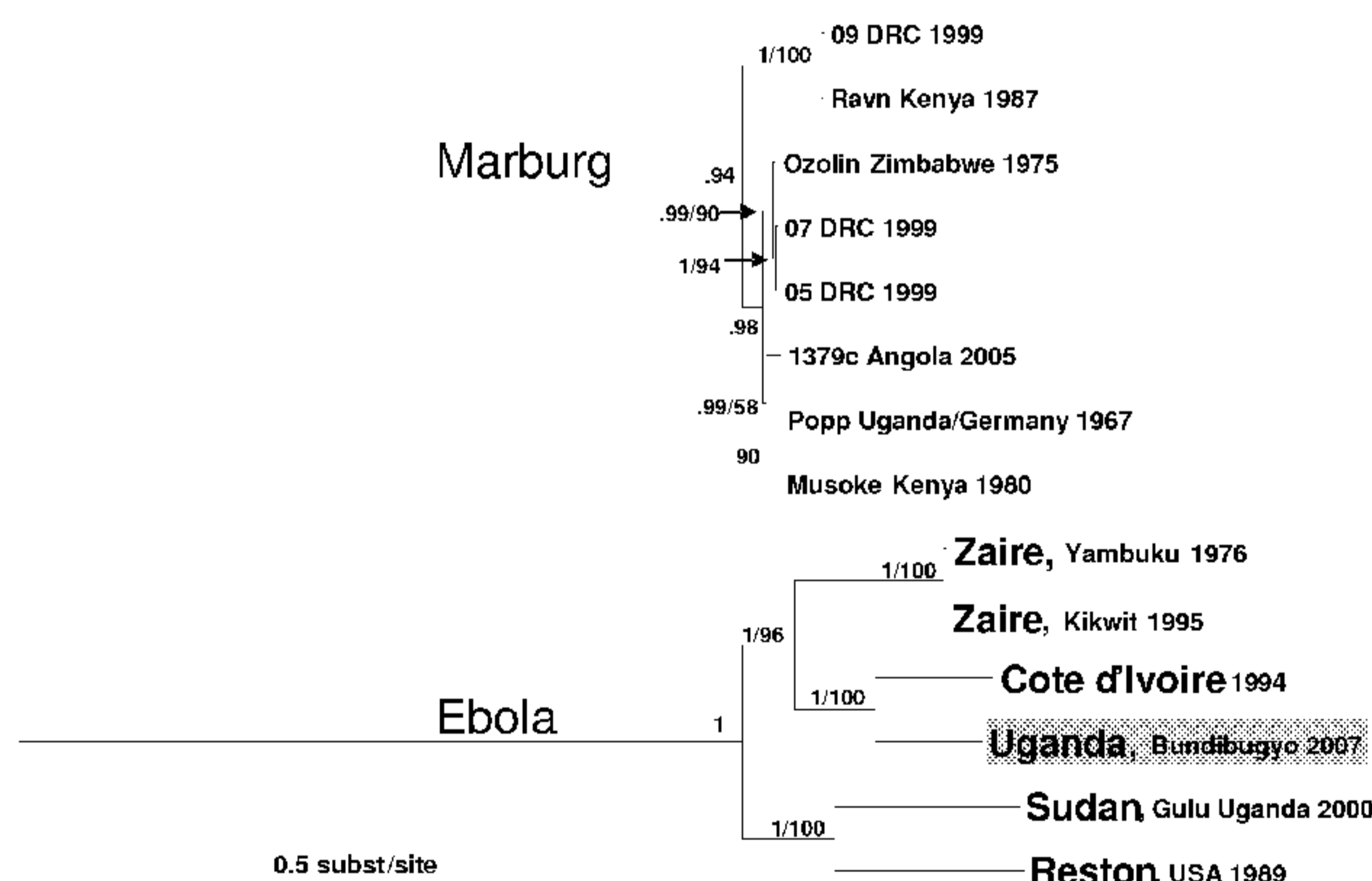


Fig. 1

(57) Abstract: Compositions and methods including and related to the Ebola Bundibugyo virus (EboBun) are provided. Compositions are provided that are operable as immunogens to elicit and immune response or protection from EboBun challenge in a subject such as a primate. Inventive methods are directed to detection and treatment of EboBun infection.

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HUMAN EBOLA VIRUS SPECIES AND COMPOSITIONS AND METHODS THEREOF

DEPOSIT STATEMENT

[0001] The invention provides the isolated human Ebola (hEbola) viruses denoted as Bundibugyo (EboBun) deposited with the Centers for Disease Control and Prevention (“CDC”;
5 Atlanta, Georgia, United States of America) on November 26, 2007 and accorded an accession number 200706291. This deposit was not made to an International Depository Authority (IDA) as established under the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure, and is a non-Budapest treaty deposit. The deposited organism is not acceptable by American Type Culture Collection (ATCC), Manassas,
10 Virginia, an International Depository Authority (IDA) as established under the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. Samples of the stated Deposit Accession No. 200706291 will be made available to approved facilities for thirty years from the date of deposit, and for the lifetime of the patent issuing from, or claiming priority to this application.

15

RELATED APPLICATIONS

[0002] This application claims priority benefit of U.S. Provisional Application 61/108,175 filed 24 October 2008; the contents of which are hereby incorporated by reference.

FIELD OF THE INVENTION

[0003] The invention is related to compositions and methods directed to a novel species of
20 human Ebola (hEbola) virus.

BACKGROUND OF THE INVENTION

[0004] The family *Filoviridae* consists of two genera, *Marburgvirus* and *Ebolavirus*, which have likely evolved from a common ancestor¹. The genus *Ebolavirus* includes four species: *Zaire*, *Sudan*, *Reston* and *Côte d’Ivoire (Ivory Coast) ebolaviruses*, which have, with the exception of
25 *Reston* and *Côte d’Ivoire ebolaviruses*, been associated with large hemorrhagic fever (HF) outbreaks in Africa with high case fatality (53-90%)².

[0005] Viruses of each species have genomes that are at least 30-40% divergent from one another, a level of diversity that presumably reflects differences in the ecologic niche they occupy

and in their evolutionary history. Identification of the natural reservoir of ebolaviruses remains somewhat elusive, although recent PCR and antibody data suggest that three species of arboreal fruit bats may be carriers of *Zaire ebolavirus*³. No data has yet been published to suggest reservoirs for the *Sudan*, *Reston* and *Côte d'Ivoire ebolavirus* species. However, a cave-dwelling fruit bat has
5 been recently implicated as a natural host for marburgvirus^{4, 5}, supporting the hypothesis that different bat species may be the reservoir hosts for the various filoviruses.

[0006] Filovirus outbreaks are sporadic, sometimes interspersed by years or even decades of no apparent disease activity. The last new species of ebolavirus was discovered 14 years ago (1994), in Cote d'Ivoire (Ivory Coast), and involved a single non-fatal case, a veterinarian who performed an
10 autopsy on an infected chimpanzee found in the Tai Forest⁶. No further disease reports have been associated with *Côte d'Ivoire ebolavirus*, in contrast to *Zaire* and *Sudan ebolaviruses* which have each caused multiple large outbreaks over the same time period.

[0007] In late November 2007, HF cases were reported in the townships of Bundibugyo and Kikyoo in Bundibugyo District, Western Uganda. The outbreak continued through January 2008, and
15 resulted in approximately 149 cases and 37 deaths². Laboratory investigation of the initial 29 suspect-case blood specimens by classic methods (antigen capture, IgM and IgG ELISA) and a recently developed random-primed pyrosequencing approach identified this to be an Ebola HF outbreak associated with a new discovered ebolavirus species. These specimens were negative when initially tested with highly sensitive real-time RT-PCR assays specific for all known *Zaire* and
20 *Sudan ebolaviruses* and *Marburg viruses*. This new species is referred to herein as "the Bundibugyo species", abbreviated "EboBun".

[0008] Accordingly, compositions and methods directed to the new Ebola virus species are described herein and the most closely related Ebola Ivory Coast species, which compositions and methods are useful for diagnosis and prevention of human Ebola virus infection; including related
25 vaccine development, and prevention of hemorrhagic fever in a human population.

SUMMARY OF THE INVENTION

[0009] The present invention is based upon the isolation and identification of a new human Ebola virus species, EboBun. EboBun was isolated from the patients suffering from hemorrhagic fever in a recent outbreak in Uganda. The isolated virus is a member of the Filoviridae family, a
30 family of negative sense RNA viruses. Accordingly, the invention relates to the isolated EboBun virus that morphologically and phylogenetically relates to known members filoviridae.

[0010] In one aspect, the invention provides the isolated EboBun virus deposited with the Centers for Disease Control and Prevention (“CDC”; Atlanta, Georgia, United States of America) on November 26, 2007 and accorded an accession number 200706291, as stated in the paragraph entitled “DEPOSIT STATEMENT” *supra*.

5 [0011] In another aspect, the invention provides an isolated hEbola EboBun virus comprising a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of: a) a nucleotide sequence set forth in SEQ ID NO: 1; b) a nucleotide sequence that hybridizes to the sequence set forth in SEQ ID NO: 1 under stringent conditions; and c) a nucleotide sequence that has at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the SEQ ID NO:
10 1. In another aspect, the invention provides the complete genomic sequence of the hEbola virus EboBun.

[0012] In a related aspect, the invention provides nucleic acid molecules isolated from EboBun, or fragments thereof.

[0013] In another aspect, the invention provides proteins or polypeptides that are isolated from
15 the EboBun, including viral proteins isolated from cells infected with the virus but not present in comparable uninfected cells; or fragments thereof. In one embodiment of the present invention, the amino acid sequences of the proteins or polypeptides are set forth in SEQ ID NOS: 2-9 and 59, or fragments thereof.

[0014] In a related aspect, the invention provides an isolated polypeptide encoded by the nucleic
20 acid molecule of the inventive hEbola EboIC (Sequence ID No. 10) virus described above.

[0015] In another aspect, the invention provides an isolated hEbola EboIC virus comprising a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of: a) a nucleotide sequence set forth in SEQ ID NO: 10; b) a nucleotide sequence that hybridizes to the sequence set forth in SEQ ID NO:10 under stringent conditions; and c) a nucleotide sequence that
25 has at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the SEQ ID NO: 10. In another aspect, the invention provides the complete genomic sequence of the hEbola virus EboIC.

[0016] In a related aspect, the invention provides nucleic acid molecules isolated from EboIC, or fragments thereof.

30 [0017] In another aspect, the invention provides proteins or polypeptides that are isolated from the EboIC, including viral proteins isolated from cells infected with the virus but not present in comparable uninfected cells; or fragments thereof. In one embodiment of the present invention, the

amino acid sequences of the proteins or polypeptides are set forth in SEQ ID NOs: 11-19, or fragments thereof.

[0018] In a related aspect, the invention provides an isolated polypeptide encoded by the nucleic acid molecule of the inventive hEbola EboIC virus described above.

5 **[0019]** In other aspects, the invention relates to the use of the isolated hEbola virus for diagnostic and therapeutic methods based on EbBun, EboIC, or a combination thereof. In one embodiment, the invention provides a method of detecting in a biological sample an antibody immunospecific for the genus of West Afrin Ebola Species constituting hEbola EbBun and EboIC virus using at least one the inventive isolated hEbola virus described herein, or any of the inventive
10 proteins or polypeptides as described herein. In another specific embodiment, the invention provides a method of screening for an antibody which immunospecifically binds and neutralizes hEbola EboBun. Such an antibody is useful for a passive immunization or immunotherapy of a subject infected with hEbola.

[0020] In another aspect, the invention provides an isolated antibody or an antigen-binding
15 fragment thereof which immunospecifically binds to the hEbola virus of the invention described above.

[0021] In other aspects, the invention provides methods for detecting the presence, activity or expression of the clade of Bundibungyo-Ivory Coast hEbola virus in a biological material, such as cells, blood, saliva, urine, feces and so forth; and specifically at least one of EbBun or EboIC.

20 **[0022]** In a related aspect, the invention provides a method for detecting the presence of the inventive hEbola virus described above in a biological sample, the method includes (a) contacting the sample with an agent that selectively binds to a West African hEbola virus; and (b) detecting whether the compound binds to the West African hEbola virus in the sample.

[0023] In another aspect, the invention provides a method for detecting the presence of the
25 inventive polypeptide described above, in a biological sample, said method includes (a) contacting the biological sample with an agent that selectively binds to the polypeptide; and (b) detecting whether the agent binds to the polypeptide in the sample. In another aspect, the invention provides a method for detecting the presence of a first nucleic acid molecule derived from the inventive hEbola virus described above in a biological sample, the method comprising: (a) contacting the biological
30 sample with an agent that selectively binds to the polypeptide; and (b) detecting whether the agent binds to the polypeptide in the sample.

[0024] In another aspect, the invention provides a method for propagating the hEbola virus in host cells comprising infecting the host cells with the inventive isolated hEbola virus described above, culturing the host cells to allow the virus to multiply, and harvesting the resulting virions. Also provided by the present invention are host cells infected with the inventive hEbola virus
5 described above.

[0025] In another aspect, the invention provides a method of detecting in a biological sample the presence of an antibody that immunospecifically binds hEbola virus, the method comprising: (a) contacting the biological sample with the inventive host cell host described above; and (b) detecting the antibody bound to the cell.

10 [0026] In another aspect, the invention provides vaccine preparations, comprising the inventive hEbola virus, including recombinant and chimeric forms of the virus, nucleic acid molecules comprised by the virus, or protein subunits of the virus. The invention also provides a vaccine formulation comprising a therapeutically or prophylactically effective amount of the inventive hEbola virus described above, and a pharmaceutically acceptable carrier. In one embodiment, the
15 invention provides a vaccine formulation comprising a therapeutically or prophylactically effective amount of a protein extract of the inventive hEbola virus described above, or a subunit thereof; and a pharmaceutically acceptable carrier. In another, the invention provides a vaccine formulation comprising a therapeutically or prophylactically effective amount of a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO: 1 or a complement thereof, and a
20 pharmaceutically acceptable carrier. In another, the invention provides a vaccine formulation comprising a therapeutically or prophylactically effective amount of a nucleic acid molecule comprising any of inventive the nucleotide sequences as described above, or a complement thereof, and a pharmaceutically acceptable carrier.

[0027] In a related aspect, the invention provides an immunogenic formulation comprising an
25 immunogenically effective amount of the inventive hEbola virus described above, and a pharmaceutically acceptable carrier. In another related aspect, the invention provides an immunogenic formulation comprising an immunogenically effective amount of a protein extract of the inventive hEbola virus described above or a subunit thereof, and a pharmaceutically acceptable carrier. In another related aspect, the invention provides an immunogenic formulation comprising an
30 immunogenically effective amount of a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO: 1 or a complement thereof, and a pharmaceutically acceptable carrier. In another related aspect, the invention provides an immunogenic formulation comprising an immunogenically

effective amount of a nucleic acid molecule comprising the inventive nucleotide sequence as described above or a complement thereof, and a pharmaceutically acceptable carrier. In another related aspect, the invention provides an immunogenic formulation comprising an immunogenically effective amount of any of the inventive polypeptides described above.

5 [0028] In another aspect, the present invention provides pharmaceutical compositions comprising antiviral agents of the present invention and a pharmaceutically acceptable carrier. In a specific embodiment, the antiviral agent of the invention is an antibody that immunospecifically binds hEbola virus or any hEbola epitope. In another specific embodiment, the antiviral agent is a polypeptide or protein of the present invention or nucleic acid molecule of the invention.

10 [0029] In a related aspect, the invention provides a pharmaceutical composition comprising a prophylactically or therapeutically effective amount of an anti-hEbola EboBun agent and a pharmaceutically acceptable carrier.

[0030] The invention also provides kits containing compositions and formulations of the present invention. Thus, in another aspect, the invention provides a kit comprising a container containing
15 the inventive immunogenic formulation described above. In another aspect, the invention provides a kit comprising a container containing the inventive vaccine formulation described above. In another, the invention provides a kit comprising a container containing the inventive pharmaceutical composition described above. In another, the invention provides a kit comprising a container containing the inventive vaccine formulation described above. In another, the invention provides a
20 method for identifying a subject infected with the inventive hEbola virus described above, comprising: (a) obtaining total RNA from a biological sample obtained from the subject; (b) reverse transcribing the total RNA to obtain cDNA; and (c) amplifying the cDNA using a set of primers derived from a nucleotide sequence of the inventive hEbola virus described above.

[0031] The invention further relates to the use of the sequence information of the isolated virus
25 for diagnostic and therapeutic methods.

[0032] In another aspect, the present invention provides methods for screening antiviral agents that inhibit the infectivity or replication of hEbola virus or variants thereof.

[0033] The invention further provides methods of preparing recombinant or chimeric forms of hEbola.

BRIEF DESCRIPTION OF THE DRAWINGS

[0034] FIG. 1 represents a Phylogenetic tree comparing full-length genomes of Ebolavirus and Marburg virus by Bayesian analysis;

[0035] FIG. 2 represents an alignment of genomes of novel hEbola EboBun (SEQ ID NO: 1) referred to below as “Ebola Bundibugyo” or “EboBun”, and hEbola Zaire (SEQ ID NO: 20); referred to below as “Ebola Zaire ‘76” or “EboZ” and hEbola Ivory Coast (SEQ ID NO: 10) also referred to below as “EboIC”.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0036] It is to be understood that the present invention is not limited to particular embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

[0037] Due to the sequence divergence of EboBun relative to all previously recognized ebolaviruses, the present invention has utility in design of diagnostic assays to monitor Ebola HF disease in humans and animals, and develop effective antivirals and vaccines.

[0038] The EboBun virus of the present invention is genetically distinct, differing by more than 30% at the genome level from all other known ebolavirus species. The unique nature of this virus created challenges for traditional filovirus molecular based diagnostic assays and genome sequencing approaches. Instead, over 70% of the virus genome was sequenced using a recently developed random-primed pyrosequencing approach which allowed the rapid development of molecular detection assay which were deployed in the disease outbreak response. This random-primed pyrosequencing draft sequence allowed faster completion of the whole genome sequence using traditional primer walking approach and confirmation that the EboBun virus represented a new ebolavirus species.

Definitions

[0039] The definitions herein provided are operative throughout the entire description of the invention set forth herein, including the Summary of the Invention.

[0040] The term “an antibody or an antibody fragment that immunospecifically binds a polypeptide of the invention” as used herein refers to an antibody or a fragment thereof that immunospecifically binds to the polypeptide encoded by the nucleotide sequence of SEQ ID NO: 1 (EboBun), or a fragment thereof, and does not non-specifically bind to other polypeptides. An antibody or a fragment thereof that immunospecifically binds to the polypeptide of the invention

may cross-react with other antigens. Preferably, an antibody or a fragment thereof that immunospecifically binds to a polypeptide of the invention does not cross-react with other antigens. An antibody or a fragment thereof that immunospecifically binds to the polypeptide of the invention can be identified by, for example, immunoassays or other techniques known to those skilled in the art, or otherwise as described herein.

[0041] An “isolated” or “purified” peptide or protein is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the protein is derived, or substantially free of chemical precursors or other chemicals when chemically synthesized. The language “substantially free of cellular material” includes preparations of a polypeptide/protein in which the polypeptide/protein is separated from cellular components of the cells from which it is isolated or recombinantly produced. Thus, a polypeptide/protein that is substantially free of cellular material includes preparations of the polypeptide/protein having less than about 30%, 20%, 10%, 5%, 2.5%, or 1% (by dry weight) of contaminating protein. When the polypeptide/protein is recombinantly produced, it is also preferably substantially free of culture medium, i.e., culture medium represents less than about 20%, 10%, or 5% of the volume of the protein preparation. When polypeptide/protein is produced by chemical synthesis, it is preferably substantially free of chemical precursors or other chemicals, i.e., it is separated from chemical precursors or other chemicals which are involved in the synthesis of the protein. Accordingly, such preparations of the polypeptide/protein have less than about 30%, 20%, 10%, 5% (by dry weight) of chemical precursors or compounds other than polypeptide/protein fragment of interest. In a preferred embodiment of the present invention, polypeptides/proteins are isolated or purified.

[0042] An “isolated” nucleic acid molecule is one which is separated from other nucleic acid molecules which are present in the natural source of the nucleic acid molecule. Moreover, an “isolated” nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized. In a preferred embodiment of the invention, nucleic acid molecules encoding polypeptides/proteins of the invention are isolated or purified. The term “isolated” nucleic acid molecule does not include a nucleic acid that is a member of a library that has not been purified away from other library clones containing other nucleic acid molecules.

[0043] The term “portion” or “fragment” as used herein includes the specified fragment lengths, and all integers in between, inclusive of the specified end points in a specified range, and inclusive of any length up to the full length of a protein, polypeptide, or nucleic acid.

[0044] The term “having a biological activity of the protein” or “having biological activities of the polypeptides of the invention” refers to the characteristics of the polypeptides or proteins having a common biological activity, similar or identical structural domain, and/or having sufficient amino acid identity to the polypeptide encoded by the nucleotide sequence of SEQ ID NO: 1 (EboBun). Such common biological activities of the polypeptides of the invention include antigenicity and immunogenicity.

[0045] The term “under stringent condition” refers to hybridization and washing conditions under which nucleotide sequences having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to each other remain hybridized to each other. Such hybridization conditions are described in, for example but not limited to, Current Protocols in Molecular Biology, John Wiley & Sons, NY (1989), 6.3.1-6.3.6.; Basic Methods in Molecular Biology, Elsevier Science Publishing Co., Inc., NY (1986), pp. 75-78, and 84-87; and Molecular Cloning, Cold Spring Harbor Laboratory, NY (1982), pp. 387-389, and are well known to those skilled in the art. A preferred, non-limiting example of stringent hybridization conditions is hybridization in 6 x sodium chloride/sodium citrate (SSC), 0.5% SDS at about 68°C followed by one or more washes in 2 x SSC, 0.5% SDS at room temperature. Another preferred, non-limiting example of stringent hybridization conditions is hybridization in 6 x SSC at about 45°C, followed by one or more washes in 0.2 x SSC, 0.1% SDS at about 50-65°C.

[0046] The term “variant” as used herein refers either to a naturally occurring genetic mutant of hEbola EboBun, or hEbola EboIC, or a recombinantly prepared variation of these hEbola species, each of which contain one or more mutations in its genome compared to the hEbola of SEQ ID NO: 1 or 10. The term “variant” may also refer either to a naturally occurring variation of a given peptide or a recombinantly prepared variation of a given peptide or protein in which one or more amino acid residues have been modified by amino acid substitution, addition, or deletion.

[0047] “Homology” refers to sequence similarity or, alternatively, sequence identity, between two or more polynucleotide sequences or two or more polypeptide sequences.

[0048] The terms “percent identity” and “% identity,” as applied to polynucleotide sequences, refer to the percentage of identical nucleotide matches between at least two polynucleotide sequences aligned using a standardized algorithm. Such an algorithm may insert, in a standardized

and reproducible way, gaps in the sequences being compared in order to optimize alignment between two sequences, and therefore achieve a more meaningful comparison of the two sequences.

[0049] Percent identity between polynucleotide sequences may be determined using one or more computer algorithms or programs known in the art or described herein. For example, percent
5 identity can be determined using the default parameters of the CLUSTAL V algorithm as incorporated into the MEGALIGN version 3.12e sequence alignment program. This program is part of the LASERGENE software package, a suite of molecular biological analysis programs (DNASTAR, Madison, Wis.). CLUSTAL V is described in Higgins, D. G. and P. M. Sharp (1989; CABIOS 5:151-153) and in Higgins, D. G. et al. (1992; CABIOS 8:189-191). For pairwise
10 alignments of polynucleotide sequences, the default parameters are set as follows: Ktuple=2, gap penalty=5, window=4, and “diagonals saved”=4. The “weighted” residue weight table is selected as the default.

[0050] Alternatively, a suite of commonly used and freely available sequence comparison algorithms which can be used is provided by the National Center for Biotechnology Information
15 (NCBI) Basic Local Alignment Search Tool (BLAST) (Altschul, S. F. et al. (1990) J. Mol. Biol. 215:403-410), which is available from several sources, including the NCBI, Bethesda, Md., and on the NCBI World Wide Web site available on the Internet. The BLAST software suite includes various sequence analysis programs including “blastn,” that is used to align a known polynucleotide sequence with other polynucleotide sequences from a variety of databases. Also available is a tool
20 called “BLAST 2 Sequences” that is used for direct pairwise comparison of two nucleotide sequences. “BLAST 2 Sequences” can be accessed and used interactively on the Internet via the NCBI World Wide Web site as well. The “BLAST 2 Sequences” tool can be used for both blastn and blastp (discussed below). BLAST programs are commonly used with gap and other parameters set to default settings. For example, to compare two nucleotide sequences, one may use blastn with
25 the “BLAST 2 Sequences” tool Version 2.0.12 (Apr. 21, 2000) set at default parameters. Such default parameters may be, for example: Matrix:BLOSUM62; Reward for match: 1; Penalty for mismatch: -2; Open Gap: 5 and Extension Gap: 2 penalties; Gap x drop-off: 50; Expect: 10; Word Size: 11; Filter: on.

[0051] Percent identity may be measured over the length of an entire defined sequence, for
30 example, as defined by a particular SEQ ID number, or may be measured over a shorter length, for example, over the length of a fragment taken from a larger, defined sequence, for instance, a fragment of at least 20, at least 30, at least 40, at least 50, at least 70, at least 100, or at least 200

contiguous nucleotides. Such lengths are exemplary only, and it is understood that any fragment length supported by the sequences shown herein, in the tables, figures, or sequence listing, may be used to describe a length over which percentage identity may be measured.

[0052] The phrases “percent identity” and “% identity”, as applied to polypeptide sequences, refer to the percentage of identical residue matches between at least two polypeptide sequences aligned using a standardized algorithm. Methods of polypeptide sequence alignment are well known. Some alignment methods take into account conservative amino acid substitutions. Such conservative substitutions, explained in more detail above, generally preserve the charge and hydrophobicity at the site of substitution, thus preserving the structure (and therefore function) of the polypeptide. The phrases “percent similarity” and “% similarity”, as applied to polypeptide sequences, refer to the percentage of residue matches, including identical residue matches and conservative substitutions, between at least two polypeptide sequences aligned using a standardized algorithm. In contrast, conservative substitutions are not included in the calculation of percent identity between polypeptide sequences.

[0053] Percent identity between polypeptide sequences may be determined using the default parameters of the CLUSTAL V algorithm as incorporated into the MEGALIGN version 3.12e sequence alignment program (described and referenced above). For pairwise alignments of polypeptide sequences using CLUSTAL V, the default parameters are set as follows: Ktuple=1, gap penalty=3, window=5, and “diagonals saved”=5. The PAM250 matrix is selected as the default residue weight table.

[0054] Alternatively the NCBI BLAST software suite may be used. For example, for a pairwise comparison of two polypeptide sequences, one may use the “BLAST 2 Sequences” tool Version 2.0.12 (Apr. 21, 2000) with blastp set at default parameters. Such default parameters may be, for example: Matrix: BLOSUM62; Open Gap: 11 and Extension Gap: 1 penalties; Gap x drop-off: 50; Expect: 10; Word Size: 3; Filter: on.

[0055] Percent identity may be measured over the length of an entire defined polypeptide sequence, for example, as defined by a particular SEQ ID number, or may be measured over a shorter length, for example, over the length of a fragment taken from a larger, defined polypeptide sequence, for instance, a fragment of at least 15, at least 20, at least 30, at least 40, at least 50, at least 70 or at least 150 contiguous residues. Such lengths are exemplary only, and it is understood that any fragment length supported by the sequences shown herein, in the tables, figures or sequence listing, may be used to describe a length over which percentage identity may be measured.

[0056] The term “agent” encompasses any chemical, biochemical, or biological molecule; such as small molecules, proteins, polypeptides, antibodies, nucleic acid molecules including DNA or RNA, and the like.

Methods and compositions related to the inventive hEbola

5 **[0057]** The present invention is based upon the isolation and identification of a new human Ebola virus species, EboBun and the sequencing of the only other known West African Ebola species EboIC. EboBun was isolated from the patients suffering from hemorrhagic fever in a recent outbreak in Uganda. The isolated virus is a member of the Filoviridae family, a family of negative
10 morphologically and phylogenetically relates to known members filoviridae.

[0058] In another aspect, the invention provides an isolated hEbola virus including a nucleic acid molecule with a nucleotide sequence that is preferably: a) a nucleotide sequence set forth in SEQ ID NO: 1; b) a nucleotide sequence that hybridizes to the sequence set forth in SEQ ID NO: 1 under stringent conditions; or c) a nucleotide sequence that has at least 70%, 75%, 80%, 85%, 90%,
15 95%, 96%, 97%, 98%, or 99% identity to the SEQ ID NO: 1. In one embodiment of the present invention, the hEbola virus is killed. In another, the virus is attenuated. In another, the infectivity of the attenuated hEbola virus is reduced. In another, the infectivity is reduced by at least 5-fold, 10-fold, 25-fold, 50-fold, 100-fold, 250-fold, 500-fold, or 10,000-fold. In another, the replication ability of the attenuated hEbola virus is reduced. In another, the replication ability of the attenuated
20 virus is reduced by at least 5-fold, 10-fold, 25-fold, 50-fold, 100-fold, 250-fold, 500-fold, 1,000-fold, or 10,000-fold. In another, the protein synthesis ability of the attenuated virus is reduced. In another, the protein synthesis ability is reduced by at least 5-fold, 10-fold, 25-fold, 50-fold, 100-fold, 250-fold, 500-fold, 1,000-fold, or 10,000-fold. In another, the assembling ability of the attenuated hEbola virus is reduced. In another, the assembling ability of the attenuated virus is reduced by at
25 least 5-fold, 10-fold, 25-fold, 50-fold, 100-fold, 250-fold, 500-fold, 1,000-fold, or 10,000-fold. In another, the cytopathic effect of the attenuated hEbola virus is reduced. In another, the cytopathic effect is reduced by at least 5-fold, 10-fold, 25-fold, 50-fold, 100-fold, 250-fold, 500-fold, 1,000-fold, or 10,000-fold.

[0059] In another aspect, the invention provides the complete genomic sequence of the hEbola virus EboBun or EboIC. In a specific embodiment, the virus includes a nucleotide sequence of SEQ ID NOs: 1 or 10, respectively.
30

[0060] In a related aspect, the invention provides nucleic acid molecules isolated from EboBun, EboIC, or fragments thereof. In one embodiment of the present invention, the isolated nucleic acid molecule includes the nucleotide sequence of SEQ ID NOs: 1 or 10, or a complement thereof. In another, the nucleic acid molecule includes a nucleotide sequence having at least 4, 5, 10, 15, 20, 25, 5 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 4600, 4700, 4800, or 4900 contiguous nucleotides of the nucleotide sequence of SEQ ID NO: 1, or a complement thereof; with the proviso that the nucleotide sequence is not comprised by the nucleotide sequence set forth in SEQ ID NO: 20 (Ebola Zaire nucleotide sequence); or at least 5000, 5500, 5600, 5700, 5800, 10 5900, 6000, 6100, 6200, 6300, 6400, 6500, or 6600 contiguous nucleotides of the nucleotide sequence of SEQ ID NOs: 1 or 10, or a complement thereof. In another embodiment, the isolated nucleic acid molecule includes a nucleotide sequence that encodes the EboBun amino acid sequence of SEQ ID NOs: 2-9 or 59, the EboIC amino acid sequence of SEQ ID NOs: 11-19, or a complement of the nucleotide sequence that encodes the EboBun amino acid sequences of SEQ ID NOs: 2-9 or 15 59 or the EboIC amino acid sequences of SEQ ID NOs: 11-19. In another, the isolated nucleic acid molecule hybridizes under stringent conditions to a nucleic acid molecule having the nucleotide sequence of SEQ ID NOs: 1 or 10 or a complement thereof, wherein the nucleic acid molecule encodes an amino acid sequence which has a biological activity exhibited by a polypeptide encoded by the nucleotide sequence of SEQ ID NOs: 1 or 10. In another, nucleic acid molecule is RNA. In 20 another, nucleic acid molecule is DNA.

[0061] In another aspect, the invention provides proteins or polypeptides that are isolated from the EboBun, including viral proteins isolated from cells infected with the virus but not present in comparable uninfected cells. In one embodiment of the present invention, the amino acid sequences of the proteins or polypeptides are set forth in SEQ ID NOs: 2-9, 59, or 11-19, or fragments thereof. 25 In one embodiment, polypeptides or proteins of the present invention have a biological activity of the protein (including antigenicity and/or immunogenicity) encoded by the sequence of SEQ ID NOs: 1 or 10. In another, the polypeptides or the proteins of the present invention have a biological activity of at least one protein having the amino acid sequence (including antigenicity and/or immunogenicity) set forth in SEQ ID NOS: 2-9, 59, or 11-19, or a fragment thereof.

30 **[0062]** In a related aspect, the invention provides an isolated polypeptide encoded by the nucleic acid molecule of the invention described above. In one embodiment of the present invention, the isolated polypeptide includes the amino acid sequence selected from the group consisting of: a) an

amino acid sequence set forth in SEQ ID NO: 2, 3, 4, 5, 6, 7, 8, or 9; 11, 12, 13, 14, 15, 16, 17, 18 or 19; and b) an amino acid sequence that has 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% homology to the amino acid sequence according to a). In another, the isolated polypeptide comprises the amino acid sequence having at least 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 150, 200, 210, 220, 230, 240 or 250 contiguous amino acid residues of the amino acid sequence of SEQ ID NOs: 5 or 18 (VP24); 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 150, 200, 210, 220, 230, 240, 250, 260, 270, 280 contiguous amino acid residues of the amino acid sequence of SEQ ID NOs: 6 or 17 (VP30); 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 310, or 320 contiguous amino acid residues of the amino acid sequence of SEQ ID NOs: 8 or 13 (VP40); 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 310, 320, 330, or 340 contiguous amino acid residues of the amino acid sequence of SEQ ID NOs: 7 or 12 (VP35); 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 310, 320, 330, 340, 350, 360, or 370 contiguous amino acid residues of the amino acid sequence of SEQ ID NOs: 4 or 15 (SGP); 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 310, 320, 330, 340, 350, 360, or 370 contiguous amino acid residues of the amino acid sequence of SEQ ID NOs: 59 or 16 (SSGP); 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, 450, 500, 550, 600, 610, 620, 630, 640, 650, 660, or 670 contiguous amino acid residues of the amino acid sequence of SEQ ID NOs: 9 or 14 (GP); 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, 450, 500, 550, 600, 650, 700, 710, 720, or 730 contiguous amino acid residues of the amino acid sequence of SEQ ID NOs: 3 or 11 (NP); or 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, 1250, 1300, 1350, 1400, 1450, 1500, 1550, 1600, 1650, 1700, 1750, 1800, 1850, 1900, 1950, 2000, 2050, 2100, 2150, 2160, 2170, 2180, 2190, or 2200 contiguous amino acid residues of the amino acid sequence of SEQ ID NOs: 2 or 19 (L).

[0063] In other aspects, the invention relates to the use of an isolated West African hEbola virus for diagnostic and therapeutic methods. In one embodiment, the invention provides a method of detecting in a biological sample an antibody immunospecific for the hEbola virus using the inventive isolated hEbola virus described herein, or any of the inventive proteins or polypeptides as described herein. In another specific embodiment, the invention provides a method of screening for an antibody which immunospecifically binds and neutralizes hEbola EboBun or EboIC or a

combination thereof. Such an antibody is useful for a passive immunization or immunotherapy of a subject infected with hEbola.

[0064] In another aspect, the invention provides an isolated antibody or an antigen-binding fragment thereof which immunospecifically binds to a West African genus hEbola virus of the invention described above, and illustratively including EboBun or EboIC. In one embodiment of the present invention, the isolated antibody or an antigen-binding fragment thereof neutralizes a West African genus hEbola virus. In another, the isolated antibody or an antigen-binding fragment thereof immunospecifically binds to the inventive polypeptide described above. The invention further provides antibodies that specifically bind a polypeptide of the invention encoded by the nucleotide sequence of SEQ ID NOs: 1 (EboBun) or 10 (EboIC), a fragment thereof, or encoded by a nucleic acid comprising a nucleotide sequence that hybridizes under stringent conditions to the nucleotide sequence of SEQ ID NOs: 1 (EboBun) or 10 (EboIC) and/or any hEbola EboBun epitope, having one or more biological activities of a polypeptide of the invention. These polypeptides include those shown in SEQ ID NOs: 2-9, 59, and 11-19. Such antibodies include, but are not limited to, polyclonal, monoclonal, bi-specific, multi-specific, human, humanized, chimeric antibodies, single chain antibodies, Fab fragments, F(ab')₂ fragments, disulfide-linked Fvs, intrabodies and fragments containing either a VL or VH domain or even a complementary determining region (CDR) that specifically binds to a polypeptide of the invention.

[0065] In other aspects, the invention provides methods for detecting the presence, activity or expression of the hEbola virus of the invention in a biological material, such as cells, blood, saliva, urine, and so forth. The increased or decreased activity or expression of the hEbola virus in a sample relative to a control sample can be determined by contacting the biological material with an agent which can detect directly or indirectly the presence, activity or expression of the hEbola virus. In one embodiment of the present invention, the detecting agents are the antibodies or nucleic acid molecules of the present invention. Antibodies of the invention can also be used to treat hemorrhagic fever.

[0066] In a related aspect, the invention provides a method for detecting the presence of the inventive hEbola virus described above in a biological sample, the method comprising: (a) contacting the sample with an agent that selectively binds to the hEbola virus; and (b) detecting whether the compound binds to the hEbola virus in the sample. In one embodiment of the present invention, the biological sample is selected from the group consisting of cells; blood; serum; plasma; feces; rectal, vaginal and conjunctival swabs. In another, the agent that binds to the virus is an

antibody. In another, the agent that binds to the virus is a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO: 1 or a complement thereof. In another, the agent that binds to the virus is a nucleic acid molecule comprising a nucleotide sequence having at least 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 4600, 4700, 4800, 4900, 5000, 5500, 5600, 5700, 5800, 5900, 6000, 6100, 6200, 6300, 6400, 6500, or 6600 contiguous nucleotides of the nucleotide sequence of SEQ ID NOs: 1 or 10, or a complement thereof.

[0067] In another aspect, the invention provides a method for detecting the presence of the inventive polypeptide described above, in a biological sample, the method comprising: (a) contacting the biological sample with an agent that selectively binds to the polypeptide; and (b) detecting whether the agent binds to the polypeptide in the sample. In one embodiment of the present invention, the biological sample is selected from the group consisting of cells; blood; serum; plasma; feces; rectal, vaginal and conjunctival swabs. In another, the agent that binds to the polypeptide is an antibody or an antigen-binding fragment thereof.

[0068] In another aspect, the invention provides a method for detecting the presence of a first nucleic acid molecule derived from the inventive hEbola virus described above in a biological sample, the method includes (a) contacting the biological sample with an agent that selectively binds to the nucleic acid; and (b) detecting whether the agent binds to the nucleotide in the sample. In one embodiment of the present invention, the agent that binds to the first nucleic acid molecule is a second nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO: 1 or a complement thereof. In another, the second nucleic acid molecule comprises at least 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 4600, 4700, 4800, 4900, 5000, 5500, 5600, 5700, 5800, 5900, 6000, 6100, 6200, 6300, 6400, 6500, or 6600 contiguous nucleotides of the nucleotide sequence of SEQ ID NOs: 1 or 10, or a complement thereof.

[0069] In another aspect, the invention provides a method for propagating the hEbola virus in host cells comprising infecting the host cells with an inventive isolated West African hEbola virus described above, culturing the host cells to allow the virus to multiply, and harvesting the resulting virions. Also provided by the present invention are host cells infected with the inventive hEbola virus described above. In one embodiment of the present invention, the host cell is a primate cell.

[0070] In another aspect, the invention provides a method of detecting in a biological sample the presence of an antibody that immunospecifically binds hEbola virus, the method includes: (a)

contacting the biological sample with the inventive host cell described above; and (b) detecting the antibody bound to the cell.

[0071] In another aspect, the invention provides vaccine preparations, including the inventive hEbola virus, including recombinant and chimeric forms of the virus, nucleic acid molecules
5 comprised by the virus, or protein subunits of the virus. In one embodiment, the vaccine preparations of the present invention includes live but attenuated hEbola virus with or without pharmaceutically acceptable carriers, including adjuvants. In another, the vaccine preparations of the invention comprise an inactivated or killed hEbola EboBun virus, EboIC virus, or a combination thereof, with or without pharmaceutically acceptable carriers, including adjuvants. Such attenuated
10 or inactivated viruses may be prepared by a series of passages of the virus through the host cells or by preparing recombinant or chimeric forms of virus. Accordingly, the present invention further provides methods of preparing recombinant or chimeric forms of the inventive hEbola viruses described herein.

[0072] In another specific embodiment, the invention provides a vaccine formulation
15 comprising a therapeutically or prophylactically effective amount of the inventive hEbola virus described above, and a pharmaceutically acceptable carrier. In another, the invention provides a vaccine formulation comprising a therapeutically or prophylactically effective amount of a protein extract of the inventive hEbola virus described above, or a subunit thereof; and a pharmaceutically acceptable carrier. In another aspect, the invention provides a vaccine formulation comprising a
20 therapeutically or prophylactically effective amount of a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NOs: 1 or 10, or a complement thereof, and a pharmaceutically acceptable carrier. In another, the invention provides a vaccine formulation comprising a therapeutically or prophylactically effective amount of a nucleic acid molecule comprising any of inventive the nucleotide sequences as described above, or a complement thereof, and a
25 pharmaceutically acceptable carrier. In another aspect, the invention provides a vaccine formulation comprising a therapeutically or prophylactically effective amount of a protein extract of the inventive hEbola virus described above, or a subunit thereof; and a pharmaceutically acceptable carrier. In another aspect, the invention provides a vaccine formulation comprising a therapeutically or prophylactically effective amount of a nucleic acid molecule comprising the nucleotide sequence
30 of SEQ ID NOs: 1 or 10, or a complement thereof, and a pharmaceutically acceptable carrier. In another, the invention provides a vaccine formulation comprising a therapeutically or prophylactically effective amount of a nucleic acid molecule comprising any of inventive the

nucleotide sequences as described above, or a complement thereof, and a pharmaceutically acceptable carrier.

[0073] In yet another specific embodiment, the vaccine preparations of the present invention comprise a nucleic acid or fragment of the hEbola virus, e.g., the virus having Accession No. 200706291, or nucleic acid molecules having the sequence of SEQ ID NOs: 1 or 10, or a fragment thereof. In another, the vaccine preparations comprise a polypeptide of the invention encoded by the nucleotide sequence of SEQ ID NOs: 1 or 10 or a fragment thereof. In a specific embodiment, the vaccine preparations comprise polypeptides of the invention as shown in SEQ ID NOs: 2-9, 59, or 11-19, or encoded by the nucleotide sequence of SEQ ID NOs: 1 or 10, or a fragment thereof.

[0074] Furthermore, the present invention provides methods for treating, ameliorating, managing or preventing hemorrhagic fever by administering the vaccine preparations or antibodies of the present invention alone or in combination with adjuvants, or other pharmaceutically acceptable excipients. Furthermore, the present invention provides methods for treating, ameliorating, managing, or preventing hemorrhagic fever by administering the inventive compositions and formulations including the vaccine preparations or antibodies of the present invention alone or in combination with antivirals [e.g., amantadine, rimantadine, gancyclovir, acyclovir, ribavirin, penciclovir, oseltamivir, foscarnet zidovudine (AZT), didanosine (ddI), lamivudine (3TC), zalcitabine (ddC), stavudine (d4T), nevirapine, delavirdine, indinavir, ritonavir, vidarabine, nelfinavir, saquinavir, relenza, tamiflu, pleconaril, interferons, etc.], steroids and corticosteroids such as prednisone, cortisone, fluticasone and glucocorticoid, antibiotics, analgesics, bronchodilators, or other treatments for respiratory and/or viral infections.

[0075] In a related aspect, the invention provides an immunogenic formulation comprising an immunogenically effective amount of the inventive hEbola virus described above, and a pharmaceutically acceptable carrier.

[0076] In another related aspect, the invention provides an immunogenic formulation comprising an immunogenically effective amount of a protein extract of the inventive hEbola virus described above or a subunit thereof, and a pharmaceutically acceptable carrier.

[0077] In another related aspect, the invention provides an immunogenic formulation comprising an immunogenically effective amount of a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NOs: 1, 10, a combination thereof, or a complement thereof, and a pharmaceutically acceptable carrier.

[0078] In another related aspect, the invention provides an immunogenic formulation comprising an immunogenically effective amount of a nucleic acid molecule comprising the inventive nucleotide sequence as described above or a complement thereof, and a pharmaceutically acceptable carrier.

5 [0079] In another related aspect, the invention provides an immunogenic formulation comprising an immunogenically effective amount of any of the inventive polypeptides described above.

[0080] In another aspect, the present invention provides pharmaceutical compositions comprising antiviral agents of the present invention and a pharmaceutically acceptable carrier. In a
10 specific embodiment, the antiviral agent of the invention is an antibody that immunospecifically binds hEbola virus or any hEbola epitope. In another specific embodiment, the antiviral agent is a polypeptide or protein of the present invention or nucleic acid molecule of the invention.

[0081] In a related aspect, the invention provides a pharmaceutical composition comprising a prophylactically or therapeutically effective amount of an anti-hEbola EboBun agent and a
15 pharmaceutically acceptable carrier. In one embodiment of the present invention, the anti-hEbola EboBun agent is an antibody or an antigen-binding fragment thereof which immunospecifically binds to the hEbola virus of Deposit Accession No. 200706291, or polypeptides or protein derived therefrom. In another, the anti-hEbola agent is a nucleic acid molecule comprising the nucleotide
20 sequence of SEQ ID NOs: 1, 10, a combination thereof, or a fragment thereof. In another, the anti-hEbola agent is a polypeptide encoded by a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NOs: 1, 10, a combination thereof, or a fragment thereof having a biological activity of the polypeptide.

[0082] The invention also provides kits containing compositions and formulations of the present invention. Thus, in another aspect, the invention provides a kit comprising a container containing
25 the inventive immunogenic formulation described above.

[0083] In another aspect, the invention provides a kit includes a container containing the inventive vaccine formulation described above.

[0084] In another aspect, the invention provides a kit including a container containing the inventive pharmaceutical composition described above.

30 [0085] In another aspect, the invention provides a kit including a container containing the inventive vaccine formulation described above.

[0086] In another aspect, the invention provides a method for identifying a subject infected with the inventive hEbola virus described above, including: (a) obtaining total RNA from a biological sample obtained from the subject; (b) reverse transcribing the total RNA to obtain cDNA; and (c) amplifying the cDNA using a set of primers derived from a nucleotide sequence of the inventive hEbola virus described above.

[0087] In one embodiment of the present invention, the set of primers are derived from the nucleotide sequence of the genome of the hEbola virus of Deposit Accession No. 200706291. In another, the set of primers are derived from the nucleotide sequence of SEQ ID NOs: 1 or 10 or any of the inventive nucleotide sequences as described above, or a complement thereof.

[0088] The invention further relates to the use of the sequence information of the isolated virus for diagnostic and therapeutic methods. In a specific embodiment, the invention provides nucleic acid molecules which are suitable for use as primers consisting of or including the nucleotide sequence of SEQ ID NOs: 1 or 10, or a complement thereof, or at least a portion of the nucleotide sequence thereof. In another specific embodiment, the invention provides nucleic acid molecules which are suitable for hybridization to the inventive hEbola nucleic acid; including, but not limited to PCR primers, Reverse Transcriptase primers, probes for Southern analysis or other nucleic acid hybridization analysis for the detection of hEbola nucleic acids, e.g., consisting of or including the nucleotide sequence of SEQ ID NOs: 1, 10 a combination thereof, a complement thereof, or a portion thereof. The invention further encompasses chimeric or recombinant viruses encoded in whole or in part by the nucleotide sequences.

[0089] In another aspect, the present invention provides methods for screening antiviral agents that inhibit the infectivity or replication of hEbola virus or variants thereof.

[0090] The invention further provides methods of preparing recombinant or chimeric forms of hEbola.

[0091] In another aspect, the invention provides vaccine preparations including the hEbola virus, including recombinant and chimeric forms of the virus, or subunits of the virus. The present invention encompasses recombinant or chimeric viruses encoded by viral vectors derived from the genome of the inventive hEbola virus described herein or natural variants thereof. In a specific embodiment, a recombinant virus is one derived from the hEbola virus of Deposit Accession No. 200706291. It is recognized that natural variants of the inventive hEbola viruses described herein comprise one or more mutations, including, but not limited to, point mutations, rearrangements,

insertions, deletions etc., to the genomic sequence. It is recognized that the mutations may or may not result in a phenotypic change.

[0092] In another specific embodiment, a chimeric virus of the invention is a recombinant hEbola EboBun or EboIC virus which further comprises a heterologous nucleotide sequence. In accordance with the invention, a chimeric virus may be encoded by a nucleotide sequence in which heterologous nucleotide sequences have been added to the genome or in which endogenous or native nucleotide sequences have been replaced with heterologous nucleotide sequences.

[0093] According to the present invention, the chimeric viruses are encoded by the viral vectors of the invention which further comprise a heterologous nucleotide sequence. In accordance with the present invention a chimeric virus is encoded by a viral vector that may or may not include nucleic acids that are non-native to the viral genome. In accordance with the invention a chimeric virus is encoded by a viral vector to which heterologous nucleotide sequences have been added, inserted or substituted for native or non-native sequences. In accordance with the present invention, the chimeric virus may be encoded by nucleotide sequences derived from different species or variants of hEbola virus. In particular, the chimeric virus is encoded by nucleotide sequences that encode antigenic polypeptides derived from different species or variants of hEbola virus.

[0094] A chimeric virus may be of particular use for the generation of recombinant vaccines protecting against two or more viruses (Tao et al., J. Virol. 72, 2955-2961; Durbin et al., 2000, J. Virol. 74, 6821-6831; Skiadopoulos et al., 1998, J. Virol. 72, 1762-1768 (1998); Teng et al., 2000, J. Virol. 74, 9317-9321). For example, it can be envisaged that a virus vector derived from the hEbola virus expressing one or more proteins of variants of hEbola virus including hEbola EboBun, or vice versa, will protect a subject vaccinated with such vector against infections by both the native hEbola and the variant. Attenuated and replication-defective viruses may be of use for vaccination purposes with live vaccines as has been suggested for other viruses. (See, for example, PCT WO 02/057302, at pp. 6 and 23; and United States Patent Application Publication 2008/0069838 incorporated by reference herein).

[0095] In accordance with the present invention the heterologous sequence to be incorporated into the viral vectors encoding the recombinant or chimeric viruses of the invention include sequences obtained or derived from different species or variants of hEbola.

[0096] In certain embodiments, the chimeric or recombinant viruses of the invention are encoded by viral vectors derived from viral genomes wherein one or more sequences, intergenic regions, termini sequences, or portions or entire ORF have been substituted with a heterologous or

non-native sequence. In certain embodiments of the invention, the chimeric viruses of the invention are encoded by viral vectors derived from viral genomes wherein one or more heterologous sequences have been inserted or added to the vector.

5 [0097] The selection of the viral vector may depend on the species of the subject that is to be treated or protected from a viral infection. If the subject is human, then an attenuated hEbola virus can be used to provide the antigenic sequences.

[0098] In accordance with the present invention, the viral vectors can be engineered to provide antigenic sequences which confer protection against infection by the inventive hEbola and natural variants thereof. The viral vectors may be engineered to provide one, two, three or more antigenic sequences. In accordance with the present invention the antigenic sequences may be derived from 10 the same virus, from different species or variants of the same type of virus, or from different viruses.

[0099] The expression products and/or recombinant or chimeric virions obtained in accordance with the invention may advantageously be utilized in vaccine formulations. The expression products and chimeric virions of the present invention may be engineered to create vaccines against a broad 15 range of pathogens, including viral and bacterial antigens, tumor antigens, allergen antigens, and auto antigens involved in autoimmune disorders. One way to achieve this goal involves modifying existing hEbola genes to contain foreign sequences in their respective external domains. Where the heterologous sequences are epitopes or antigens of pathogens, these chimeric viruses may be used to induce a protective immune response against the disease agent from which these determinants are 20 derived. In particular, the chimeric virions of the present invention may be engineered to create vaccines for the protection of a subject from infections with hEbola virus and variants thereof.

[00100] Thus, the present invention further relates to the use of viral vectors and recombinant or chimeric viruses to formulate vaccines against a broad range of viruses and/or antigens. The present invention also encompasses recombinant viruses including a viral vector derived from the hEbola or 25 variants thereof which contains sequences which result in a virus having a phenotype more suitable for use in vaccine formulations, e.g., attenuated phenotype or enhanced antigenicity. The mutations and modifications can be in coding regions, in intergenic regions and in the leader and trailer sequences of the virus.

[00101] The invention provides a host cell including a nucleic acid or a vector according to the 30 invention. Plasmid or viral vectors containing the polymerase components of hEbola virus are generated in prokaryotic cells for the expression of the components in relevant cell types (bacteria, insect cells, eukaryotic cells). Plasmid or viral vectors containing full-length or partial copies of the

hEbola genome will be generated in prokaryotic cells for the expression of viral nucleic acids *in vitro* or *in vivo*. The latter vectors optionally contain other viral sequences for the generation of chimeric viruses or chimeric virus proteins, optionally lack parts of the viral genome for the generation of replication defective virus, and optionally contain mutations, deletions or insertions for the generation of attenuated viruses. In addition, the present invention provides a host cell infected with hEbola virus of Deposit Accession No. 200706291,

[00102] Infectious copies of West African hEbola (being wild type, attenuated, replication-defective or chimeric) are optionally produced upon co-expression of the polymerase components according to the state-of-the-art technologies described above.

[0100] In addition, eukaryotic cells, transiently or stably expressing one or more full-length or partial hEbola proteins are optionally used. Such cells are preferably made by transfection (proteins or nucleic acid vectors), infection (viral vectors) or transduction (viral vectors) and are useful for complementation of mentioned wild type, attenuated, replication-defective or chimeric viruses.

[0101] The viral vectors and chimeric viruses of the present invention optionally modulate a subject's immune system by stimulating a humoral immune response, a cellular immune response or by stimulating tolerance to an antigen. As used herein, a subject means: humans, primates, horses, cows, sheep, pigs, goats, dogs, cats, avian species and rodents.

Formulation of Vaccines and Antivirals

[0102] In a preferred embodiment, the invention provides a proteinaceous molecule or hEbola virus specific viral protein or functional fragment thereof encoded by a nucleic acid according to the invention. Useful proteinaceous molecules are for example derived from any of the genes or genomic fragments derivable from the virus according to the invention, preferably the GP, L, NP, sGP, VP24, VP30, VP35, and VP 40 proteins described herein. Such molecules, or antigenic fragments thereof, as provided herein, are for example useful in diagnostic methods or kits and in pharmaceutical compositions such as subunit vaccines. Particularly useful are polypeptides encoded by the nucleotide sequence of SEQ ID NOs: 1 or 10; or antigenic fragments thereof for inclusion as antigen or subunit immunogen, but inactivated whole virus can also be used. Particularly useful are also those proteinaceous substances that are encoded by recombinant nucleic acid fragments of the hEbola genome, of course preferred are those that are within the preferred bounds and metes of ORFs, in particular, for eliciting hEbola specific antibody or T cell responses, whether *in vivo* (e.g.

for protective or therapeutic purposes or for providing diagnostic antibodies) or *in vitro* (e.g. by phage display technology or another technique useful for generating synthetic antibodies).

[0103] It is recognized that numerous variants, analogues, or homologues of EboBun polypeptides are within the scope of the present invention including amino acid substitutions, alterations, modifications, or other amino acid changes that increase, decrease, or do not alter the function or immunogenic propensity of the inventive immunogen or vaccine. Several post-translational modifications are similarly envisioned as within the scope of the present invention illustratively including incorporation of a non-naturally occurring amino acid(s), phosphorylation, glycosylation, sulfation, and addition of pendent groups such as biotinylation, fluorophores, lumiphores, radioactive groups, antigens, or other molecules.

[0104] Methods of expressing and purifying natural or recombinant peptides and proteins are well known in the art. Illustratively, peptides and proteins are recombinantly expressed in eukaryotic cells. Exemplary eukaryotic cells include yeast, HeLa cells, 293 cells, COS cells, Chinese hamster ovary cells (CHO), and many other cell types known in the art. Both eukaryotic and prokaryotic expression systems and cells are available illustratively from Invitrogen Corp., Carlsbad, CA. It is appreciated that cell-free expression systems are similarly operable.

[0105] In a preferred embodiment an immunogenic polypeptide is a full length EboBun protein. Preferably, an immunogen is a full length EboBun protein of SEQ ID NOs: 2-9 or 59, or EboIC SEQ ID NOs: 11-19, or a fragment thereof as described herein. Preferably, an immunogen is has a minimum of 5 amino acids. As used herein an immunogen is preferably a polypeptide. In the context of an immunogenic polypeptide the terms immunogen, polypeptide, and antigen are used interchangeably.

[0106] Modifications and changes can be made in the structure of the inventive immunogens that are the subject of the application and still obtain a molecule having similar or improved characteristics as the wild-type sequence (e.g., a conservative amino acid substitution). For example, certain amino acids are optionally substituted for other amino acids in a sequence without appreciable loss of immunogenic activity. Because it is the interactive capacity and nature of a polypeptide that defines that polypeptide's biological functional activity, certain amino acid sequence substitutions can be made in a polypeptide sequence and nevertheless obtain a polypeptide with like or improved properties. Optionally, a polypeptide is used that has less or more immunogenic activity compared to the wild-type sequence.

[0107] In making such changes, the hydrophobic index of amino acids is preferably considered. The importance of the hydrophobic amino acid index in conferring interactive biologic function on a polypeptide is generally understood in the art. It is known that certain amino acids can be substituted for other amino acids having a similar hydrophobic index or score and still result in a polypeptide with similar biological activity. Each amino acid has been assigned a hydrophobic index on the basis of its hydrophobicity and charge characteristics. Those indices are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cysteine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

[0108] It is believed that the relative hydrophobic character of the amino acid determines the secondary structure of the resultant polypeptide, which in turn defines the interaction of the polypeptide with other molecules, such as enzymes, substrates, receptors, antibodies, antigens, and the like. It is known in the art that an amino acid can be substituted by another amino acid having a similar hydrophobic index and still obtain a functionally equivalent immunogen. In such changes, the substitution of amino acids whose hydrophobic indices are within ± 2 is preferred, those within ± 1 are particularly preferred, and those within ± 0.5 are even more particularly preferred.

[0109] As outlined above, amino acid substitutions are generally based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions that take various of the foregoing characteristics into consideration are well known to those of skill in the art and include (original residue: exemplary substitution): (Ala: Gly, Ser), (Arg: Lys), (Asn: Gln, His), (Asp: Glu, Cys, Ser), (Gln: Asn), (Glu: Asp), (Gly: Ala), (His: Asn, Gln), (Ile: Leu, Val), (Leu: Ile, Val), (Lys: Arg), (Met: Leu, Tyr), (Ser: Thr), (Thr: Ser), (Tyr: Trp, Phe), and (Val: Ile, Leu). Embodiments of this disclosure thus contemplate functional or biological equivalents of a polypeptide and immunogen as set forth above. In particular, embodiments of the polypeptides and immunogens optionally include variants having about 50%, 60%, 70%, 80%, 90%, and 95% sequence identity to the polypeptide of interest.

[0110] The invention provides vaccine formulations for the prevention and treatment of infections with hEbola virus. In certain embodiments, the vaccine of the invention comprises recombinant and chimeric viruses of the hEbola virus. In certain embodiments, the virus is attenuated.

[0111] In another embodiment of this aspect of the invention, inactivated vaccine formulations are prepared using conventional techniques to “kill” the chimeric viruses. Inactivated vaccines are “dead” in the sense that their infectivity has been destroyed. Ideally, the infectivity of the virus is destroyed without affecting its immunogenicity. In order to prepare inactivated vaccines, the
5 chimeric virus may be grown in cell culture or in the allantois of the chick embryo, purified by zonal ultracentrifugation, inactivated by formaldehyde or β -propiolactone, and pooled. The resulting vaccine is usually inoculated intramuscularly or intranasally.

[0112] Inactivated viruses are optionally formulated with a suitable adjuvant in order to enhance the immunological response. Such adjuvants illustratively include but are not limited to mineral
10 gels, e.g., aluminum hydroxide; surface active substances such as lysolecithin, pluronic polyols, polyanions; peptides; oil emulsions; and potentially useful human adjuvants such as BCG and *Corynebacterium parvum*.

[0113] In another aspect, the present invention also provides DNA vaccine formulations including a nucleic acid or fragment of the inventive hEbola virus, e.g., the virus having Accession
15 No. 200706291, or nucleic acid molecules having the sequence of SEQ ID NOs: 1 or 10, or a fragment thereof. In another specific embodiment, the DNA vaccine formulations of the present invention comprise a nucleic acid or fragment thereof encoding the antibodies which immunospecifically bind hEbola viruses. In DNA vaccine formulations, a vaccine DNA comprises a viral vector, such as that derived from the hEbola virus, bacterial plasmid, or other expression
20 vector, bearing an insert including a nucleic acid molecule of the present invention operably linked to one or more control elements, thereby allowing expression of the vaccinating proteins encoded by the nucleic acid molecule in a vaccinated subject. Such vectors can be prepared by recombinant DNA technology as recombinant or chimeric viral vectors carrying a nucleic acid molecule of the present invention.

[0114] A nucleic acid as used herein refers to single- or double-stranded molecules which are
25 optionally DNA, including the nucleotide bases A, T, C and G, or RNA, including the bases A, U (substitutes for T), C, and G. The nucleic acid may represent a coding strand or its complement. Nucleic acids are optionally identical in sequence to the sequence which is naturally occurring or include alternative codons which encode the same amino acid as that which is found in the naturally
30 occurring sequence. Furthermore, nucleic acids optionally include codons which represent conservative substitutions of amino acids as are well known in the art.

[0115] As used herein, the term “isolated nucleic acid” means a nucleic acid separated or substantially free from at least some of the other components of the naturally occurring organism, for example, the cell structural components commonly found associated with nucleic acids in a cellular environment and/or other nucleic acids. The isolation of nucleic acids is illustratively
5 accomplished by techniques such as cell lysis followed by phenol plus chloroform extraction, followed by ethanol precipitation of the nucleic acids. The nucleic acids of this invention are illustratively isolated from cells according to methods well known in the art for isolating nucleic acids. Alternatively, the nucleic acids of the present invention are optionally synthesized according to standard protocols well described in the literature for synthesizing nucleic acids. Modifications to
10 the nucleic acids of the invention are also contemplated, provided that the essential structure and function of the peptide or polypeptide encoded by the nucleic acid are maintained.

[0116] The nucleic acid encoding the peptide or polypeptide of this invention is optionally part of a recombinant nucleic acid construct comprising any combination of restriction sites and/or functional elements as are well known in the art which facilitate molecular cloning and other
15 recombinant DNA manipulations. Thus, the present invention further provides a recombinant nucleic acid construct including a nucleic acid encoding a polypeptide of this invention.

[0117] Generally, it may be more convenient to employ as the recombinant polynucleotide a cDNA version of the polynucleotide. It is believed that the use of a cDNA version will provide advantages in that the size of the gene will generally be much smaller and more readily employed to
20 transfect the targeted cell than will a genomic gene, which will typically be up to an order of magnitude larger than the cDNA gene. However, the inventor does not exclude the possibility of employing a genomic version of a particular gene where desired.

[0118] As used herein, the terms “engineered” and “recombinant” cells are synonymous with “host” cells and are intended to refer to a cell into which an exogenous DNA segment or gene, such
25 as a cDNA or gene has been introduced. Therefore, engineered cells are distinguishable from naturally occurring cells which do not contain a recombinantly introduced exogenous DNA segment or gene. A host cell is optionally a naturally occurring cell that is transformed with an exogenous DNA segment or gene or a cell that is not modified. A host cell preferably does not possess a naturally occurring gene encoding RSV G protein. Engineered cells are, thus, cells having a gene or
30 genes introduced through the hand of man. Recombinant cells illustratively include those having an introduced cDNA or genomic DNA, and also include genes positioned adjacent to a promoter not naturally associated with the particular introduced gene.

[0119] To express a recombinant encoded polypeptide in accordance with the present invention one optionally prepares an expression vector that comprises a polynucleotide under the control of one or more promoters. To bring a coding sequence “under the control of” a promoter, one positions the 5' end of the translational initiation site of the reading frame generally between about 1 and 50
5 nucleotides “downstream” of (i.e., 3' of) the chosen promoter. The “upstream” promoter stimulates transcription of the inserted DNA and promotes expression of the encoded recombinant protein. This is the meaning of “recombinant expression” in the context used here.

[0120] Many standard techniques are available to construct expression vectors containing the appropriate nucleic acids and transcriptional/translational control sequences in order to achieve
10 protein or peptide expression in a variety of host-expression systems. Cell types available for expression include, but are not limited to, bacteria, such as *E. coli* and *B. subtilis* transformed with recombinant phage DNA, plasmid DNA or cosmid DNA expression vectors.

[0121] Certain examples of prokaryotic hosts illustratively include *E. coli* strain RR1, *E. coli* LE392, *E. coli* B, *E. coli* 1776 (ATCC No. 31537) as well as *E. coli* W3110 (F-, lambda-,
15 prototrophic, ATCC No. 273325); bacilli such as *Bacillus subtilis*; and other enterobacteria such as *Salmonella typhimurium*, *Serratia marcescens*, and various *Pseudomonas* species.

[0122] In general, plasmid vectors containing replicon and control sequences that are derived from species compatible with the host cell are used in connection with these hosts. The vector ordinarily carries a replication site, as well as marking sequences that are capable of providing
20 phenotypic selection in transformed cells. For example, *E. coli* is often transformed using pBR322, a plasmid derived from an *E. coli* species. Plasmid pBR322 contains genes for ampicillin and tetracycline resistance and thus provides easy means for identifying transformed cells. The pBR322 plasmid, or other microbial plasmid or phage may also contain, or be modified to contain, promoters that can be used by the microbial organism for expression of its own proteins.

25 [0123] In addition, phage vectors containing replicon and control sequences that are compatible with the host microorganism are optionally used as transforming vectors in connection with these hosts. For example, the phage lambda is optionally utilized in making a recombinant phage vector that can be used to transform host cells, such as *E. coli* LE392.

[0124] Further useful vectors include pIN vectors and pGEX vectors, for use in generating
30 glutathione S-transferase (GST) soluble fusion proteins for later purification and separation or cleavage. Other suitable fusion proteins are those with β -galactosidase, ubiquitin, or the like.

[0125] Promoters that are most commonly used in recombinant DNA construction include the β -lactamase (penicillinase), lactose and tryptophan (trp) promoter systems. While these are the most commonly used, other microbial promoters have been discovered and utilized, and details concerning their nucleotide sequences have been published, enabling those of skill in the art to ligate them functionally with plasmid vectors.

[0126] For expression in *Saccharomyces*, the plasmid YRp7, for example, is commonly used. This plasmid contains the trp1 gene, which provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example ATCC No. 44076 or PEP4-1. The presence of the trp1 lesion as a characteristic of the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan.

[0127] Suitable promoting sequences in yeast vectors illustratively include the promoters for 3-phosphoglycerate kinase or other glycolytic enzymes, such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase. In constructing suitable expression plasmids, the termination sequences associated with these genes are also preferably ligated into the expression vector 3' of the sequence desired to be expressed to provide polyadenylation of the mRNA and termination.

[0128] Other suitable promoters, which have the additional advantage of transcription controlled by growth conditions, illustratively include the promoter region for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, and the aforementioned glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization.

[0129] In addition to microorganisms, cultures of cells derived from multicellular organisms are also operable as hosts. In principle, any such cell culture is operable, whether from vertebrate or invertebrate culture. In addition to mammalian cells, these include insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus); and plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing one or more coding sequences.

[0130] In a useful insect system, *Autographica californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The isolated nucleic acid coding sequences are cloned into non-essential regions (for example the

polyhedron gene) of the virus and placed under control of an AcNPV promoter (for example, the polyhedron promoter). Successful insertion of the coding sequences results in the inactivation of the polyhedron gene and production of non-occluded recombinant virus (i.e., virus lacking the proteinaceous coat coded for by the polyhedron gene). These recombinant viruses are then used to infect *Spodoptera frugiperda* cells in which the inserted gene is expressed (e.g., U.S. Patent No. 4,215,051).

[0131] Examples of useful mammalian host cell lines include VERO and HeLa cells, Chinese hamster ovary (CHO) cell lines, W138, BHK, COS-7, 293, HepG2, NIH3T3, RIN and MDCK cell lines. In addition, a host cell is preferably chosen that modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of the encoded protein.

[0132] Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins. Appropriate cell lines or host systems are preferably chosen to ensure the correct modification and processing of the foreign protein expressed. Expression vectors for use in mammalian cells ordinarily include an origin of replication (as necessary), a promoter located in front of the gene to be expressed, along with any necessary ribosome binding sites, RNA splice sites, polyadenylation site, and transcriptional terminator sequences. The origin of replication is preferably provided either by construction of the vector to include an exogenous origin, such as may be derived from SV40 or other viral (e.g., Polyoma, Adeno, VSV, BPV) source, or may be provided by the host cell chromosomal replication mechanism. If the vector is integrated into the host cell chromosome, the latter is often sufficient.

[0133] The promoters are optionally derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter). Further, it is also possible, and may be desirable, to utilize promoter or control sequences normally associated with the desired gene sequence, provided such control sequences are compatible with the host cell systems.

[0134] A number of viral based expression systems are operable herein, for example, commonly used promoters are derived from polyoma, Adenovirus 2, Adenovirus 5, cytomegalovirus and Simian Virus 40 (SV40). The early and late promoters of SV40 virus are useful because both are obtained easily from the virus as a fragment which also contains the SV40 viral origin of replication. Smaller or larger SV40 fragments are also operable, particularly when there is included the

approximately 250 bp sequence extending from the HindIII site toward the BglII site located in the viral origin of replication.

[0135] In cases where an adenovirus is used as an expression vector, the coding sequences are preferably ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene is then optionally inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing proteins in infected hosts.

[0136] Specific initiation signals may also be required for efficient translation of the claimed isolated nucleic acid coding sequences. These signals include the ATG initiation codon and adjacent sequences. Exogenous translational control signals, including the ATG initiation codon, may additionally need to be provided. One of ordinary skill in the art would readily be capable of determining this need and providing the necessary signals. It is well known that the initiation codon must be in-frame (or in-phase) with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons are optionally of a variety of origins, both natural and synthetic. The efficiency of expression is optionally enhanced by the inclusion of appropriate transcription enhancer elements or transcription terminators.

[0137] In eukaryotic expression, one will also typically desire to incorporate into the transcriptional unit an appropriate polyadenylation site if one was not contained within the original cloned segment. Typically, the poly A addition site is placed about 30 to 2000 nucleotides “downstream” of the termination site of the protein at a position prior to transcription termination.

[0138] For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines that stably express constructs encoding proteins are engineered. Rather than using expression vectors that contain viral origins of replication, host cells are preferably transformed with vectors controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched medium, and then are switched to a selective medium. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci, which in turn can be cloned and expanded into cell lines.

[0139] A number of selection systems are illustratively used, including, but not limited, to the herpes simplex virus thymidine kinase, hypoxanthine-guanine phosphoribosyltransferase and adenine phosphoribosyltransferase genes, in tk-, hgprt- or aprt- cells, respectively. Also, antimetabolite resistance is optionally used as the basis of selection for dhfr, which confers
5 resistance to methotrexate; gpt, which confers resistance to mycophenolic acid; neo, which confers resistance to the aminoglycoside G-418; and hygro, which confers resistance to hygromycin. It is appreciated that numerous other selection systems are known in the art that are similarly operable in the present invention.

[0140] The nucleic acids encoding the peptides and polypeptides of this invention are optionally
10 administered as nucleic acid vaccines. For the purposes of vaccine delivery, a nucleic acid encoding a peptide or polypeptide of this invention is preferably in an expression vector that includes viral nucleic acid including, but not limited to, vaccinia virus, adenovirus, retrovirus and/or adeno-associated virus nucleic acid. The nucleic acid or vector of this invention is optionally in a liposome or a delivery vehicle which can be taken up by a cell via receptor-mediated or other type of
15 endocytosis. The nucleic acid vaccines of this invention are preferably in a pharmaceutically acceptable carrier or administered with an adjuvant. The nucleic acids encoding the peptides and polypeptides of this invention can also be administered to cells *in vivo* or *ex vivo*.

[0141] It is contemplated that the isolated nucleic acids of the disclosure are optionally
20 “overexpressed”, i.e., expressed in increased levels relative to its natural expression in cells of its indigenous organism, or even relative to the expression of other proteins in the recombinant host cell. Such overexpression is assessed by a variety of methods illustratively including radio-labeling and/or protein purification. However, simple and direct methods are preferred, for example, those involving SDS/PAGE and protein staining or immunoblotting, followed by quantitative analyses, such as densitometric scanning of the resultant gel or blot. A specific increase in the level of the
25 recombinant protein or peptide in comparison to the level in natural in transfected cells is indicative of overexpression, as is a relative abundance of the specific protein in relation to the other proteins produced by the host cell and, e.g., visible on a gel.

[0142] Various heterologous vectors are described for DNA vaccinations against viral
30 infections. For example, the vectors described in the following references, incorporated herein by reference, may be used to express hEbola sequences instead of the sequences of the viruses or other pathogens described; in particular, vectors described for hepatitis B virus (Michel, M. L. et al., 1995, DAN-mediated immunization to the hepatitis B surface antigen in mice: Aspects of the humoral

response mimic hepatitis B viral infection in humans, *Proc. Natl. Aca. Sci. USA* 92:5307-5311; Davis, H. L. et al., 1993, DNA-based immunization induces continuous secretion of hepatitis B surface antigen and high levels of circulating antibody, *Human Molec. Genetics* 2:1847-1851), HIV virus (Wang, B. et al., 1993, Gene inoculation generates immune responses against human immunodeficiency virus type 1, *Proc. Natl. Acad. Sci. USA* 90:4156-4160; Lu, S. et al., 1996, Simian immunodeficiency virus DNA vaccine trial in Macques, *J. Virol.* 70:3978-3991; Letvin, N. L. et al., 1997, Potent, protective anti-HIV immune responses generated by bimodal HIV envelope DNA plus protein vaccination, *Proc Natl Acad Sci USA.* 94(17):9378-83), and influenza viruses (Robinson, H L et al., 1993, Protection against a lethal influenza virus challenge by immunization with a haemagglutinin-expressing plasmid DNA, *Vaccine* 11:957-960; Ulmer, J. B. et al., Heterologous protection against influenza by injection of DNA encoding a viral protein, *Science* 259:1745-1749), as well as bacterial infections, such as tuberculosis (Tascon, R. E. et al., 1996, Vaccination against tuberculosis by DNA injection, *Nature Med.* 2:888-892; Huygen, K. et al., 1996, Immunogenicity and protective efficacy of a tuberculosis DNA vaccine, *Nature Med.*, 2:893-898), and parasitic infection, such as malaria (Sedegah, M., 1994, Protection against malaria by immunization with plasmid DNA encoding circumsporozoite protein, *Proc. Natl. Acad. Sci. USA* 91:9866-9870; Doolan, D. L. et al., 1996, Circumventing genetic restriction of protection against malaria with multigene DNA immunization: CD8+T cell-interferon .delta., and nitric oxide-dependent immunity, *J. Exper. Med.*, 1183:1739-1746).

20 **[0143]** Many methods are optionally used to introduce the vaccine formulations described above. These include, but are not limited to, oral, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, and intranasal routes. Alternatively, in a preferred embodiment the chimeric virus vaccine formulation is introduced via the natural route of infection of the pathogen for which the vaccine is designed. The DNA vaccines of the present invention are optionally administered in saline solutions by injections into muscle or skin using a syringe and needle (Wolff J. A. et al., 1990, Direct gene transfer into mouse muscle in vivo, *Science* 247:1465-1468; Raz, E., 1994, Intradermal gene immunization: The possible role of DNA uptake in the induction of cellular immunity to viruses, *c. Natl. Aca. Sci. USA* 91:9519-9523). Another way to administer DNA vaccines operable herein is called the "gene gun" method, whereby microscopic gold beads coated with the DNA molecules of interest is fired into cells (Tang, D. et al., 1992, Genetic immunization is a simple method for eliciting an immune response, *Nature* 356:152-154). For general reviews of the methods for DNA vaccines, see Robinson, H. L., 1999, DNA vaccines: basic mechanism and

immune responses (Review), *Int. J. Mol. Med.* 4(5):549-555; Barber, B., 1997, Introduction: Emerging vaccine strategies, *Seminars in Immunology* 9(5):269-270; and Robinson, H. L. et al., 1997, DNA vaccines, *Seminars in Immunology* 9(5):271-283.

5 Attenuation of hEbola Virus or Variants Thereof

[0144] The hEbola virus or variants thereof of the invention are optionally genetically engineered to exhibit an attenuated phenotype. In particular, the viruses of the invention exhibit an attenuated phenotype in a subject to which the virus is administered as a vaccine. Attenuation can be achieved by any method known to a skilled artisan. Without being bound by theory, the
10 attenuated phenotype of the viruses of the invention is caused, e.g., by using a virus that naturally does not replicate well in an intended host species, for example, by reduced replication of the viral genome, by reduced ability of the virus to infect a host cell, or by reduced ability of the viral proteins to assemble to an infectious viral particle relative to the wild type species of the virus.

[0145] The attenuated phenotypes of hEbola virus or variants thereof are optionally tested by
15 any method known to the artisan. A candidate virus, for example, is optionally tested for its ability to infect a host or for the rate of replication in a cell culture system. In certain embodiments, growth curves at different temperatures are used to test the attenuated phenotype of the virus. For example, an attenuated virus is able to grow at 35°C, but not at 39°C or 40°C. In certain embodiments, different cell lines are used to evaluate the attenuated phenotype of the virus. For example, an
20 attenuated virus may only be able to grow in monkey cell lines but not the human cell lines, or the achievable virus titers in different cell lines are different for the attenuated virus. In certain embodiments, viral replication in the respiratory tract of a small animal model, including but not limited to, hamsters, cotton rats, mice and guinea pigs, is used to evaluate the attenuated phenotypes of the virus. In other embodiments, the immune response induced by the virus, including but not
25 limited to, the antibody titers (e.g., assayed by plaque reduction neutralization assay or ELISA) is used to evaluate the attenuated phenotypes of the virus. In a specific embodiment, the plaque reduction neutralization assay or ELISA is carried out at a low dose. In certain embodiments, the ability of the hEbola virus to elicit pathological symptoms in an animal model is tested. A reduced ability of the virus to elicit pathological symptoms in an animal model system is indicative of its
30 attenuated phenotype. In a specific embodiment, the candidate viruses are tested in a monkey model for nasal infection, indicated by mucus production.

[0146] The viruses of the invention are optionally attenuated such that one or more of the functional characteristics of the virus are impaired. In certain embodiments, attenuation is measured in comparison to the wild type species of the virus from which the attenuated virus is derived. In other embodiments, attenuation is determined by comparing the growth of an attenuated virus in
5 different host systems. Thus, for a non-limiting example, hEbola virus or a variant thereof is attenuated when grown in a human host if the growth of the hEbola or variant thereof in the human host is reduced compared to the non-attenuated hEbola or variant thereof.

[0147] In certain embodiments, the attenuated virus of the invention is capable of infecting a host, is capable of replicating in a host such that infectious viral particles are produced. In
10 comparison to the wild type species, however, the attenuated species grows to lower titers or grows more slowly. Any technique known to the skilled artisan can be used to determine the growth curve of the attenuated virus and compare it to the growth curve of the wild type virus.

[0148] In certain embodiments, the attenuated virus of the invention (e.g., a recombinant or chimeric hEbola) cannot replicate in human cells as well as the wild type virus (e.g., wild type
15 hEbola) does. However, the attenuated virus can replicate well in a cell line that lacks interferon functions, such as Vero cells.

[0149] In other embodiments, the attenuated virus of the invention is capable of infecting a host, of replicating in the host, and of causing proteins of the virus of the invention to be inserted into the cytoplasmic membrane, but the attenuated virus does not cause the host to produce new infectious
20 viral particles. In certain embodiments, the attenuated virus infects the host, replicates in the host, and causes viral proteins to be inserted in the cytoplasmic membrane of the host with the same efficiency as the wild type hEbola. In other embodiments, the ability of the attenuated virus to cause viral proteins to be inserted into the cytoplasmic membrane into the host cell is reduced compared to the wild type virus. In certain embodiments, the ability of the attenuated hEbola virus to replicate in
25 the host is reduced compared to the wild type virus. Any technique known to the skilled artisan can be used to determine whether a virus is capable of infecting a mammalian cell, of replicating within the host, and of causing viral proteins to be inserted into the cytoplasmic membrane of the host.

[0150] In certain embodiments, the attenuated virus of the invention is capable of infecting a host. In contrast to the wild type hEbola, however, the attenuated hEbola cannot be replicated in the
30 host. In a specific embodiment, the attenuated hEbola virus can infect a host and can cause the host to insert viral proteins in its cytoplasmic membranes, but the attenuated virus is incapable of being replicated in the host. Any method known to the skilled artisan can be used to test whether the

attenuated hEbola has infected the host and has caused the host to insert viral proteins in its cytoplasmic membranes.

[0151] In certain embodiments, the ability of the attenuated virus to infect a host is reduced compared to the ability of the wild type virus to infect the same host. Any technique known to the skilled artisan can be used to determine whether a virus is capable of infecting a host.

[0152] In certain embodiments, mutations (e.g., missense mutations) are introduced into the genome of the virus, for example, into the sequence of SEQ ID NOs: 1 or 10, or to generate a virus with an attenuated phenotype. Mutations (e.g., missense mutations) can be introduced into the structural genes and/or regulatory genes of the hEbola. Mutations are optionally additions, substitutions, deletions, or combinations thereof. Such variant of hEbola can be screened for a predicted functionality, such as infectivity, replication ability, protein synthesis ability, assembling ability, as well as cytopathic effect in cell cultures. In a specific embodiment, the missense mutation is a cold-sensitive mutation. In another embodiment, the missense mutation is a heat-sensitive mutation. In another embodiment, the missense mutation prevents a normal processing or cleavage of the viral proteins.

[0153] In other embodiments, deletions are introduced into the genome of the hEbola virus, which result in the attenuation of the virus.

[0154] In certain embodiments, attenuation of the virus is achieved by replacing a gene of the wild type virus with a gene of a virus of a different species, of a different subgroup, or of a different variant. In another aspect, attenuation of the virus is achieved by replacing one or more specific domains of a protein of the wild type virus with domains derived from the corresponding protein of a virus of a different species. In certain other embodiments, attenuation of the virus is achieved by deleting one or more specific domains of a protein of the wild type virus.

[0155] When a live attenuated vaccine is used, its safety should also be considered. The vaccine preferably does not cause disease. Any techniques known in the art for improving vaccine safety are operable in the present invention. In addition to attenuation techniques, other techniques are optionally be used. One non-limiting example is to use a soluble heterologous gene that cannot be incorporated into the virion membrane. For example, a single copy of the soluble version of a viral transmembrane protein lacking the transmembrane and cytosolic domains thereof is used.

[0156] Various assays are optionally used to test the safety of a vaccine. For example, sucrose gradients and neutralization assays are used to test the safety. A sucrose gradient assay is optionally used to determine whether a heterologous protein is inserted in a virion. If the heterologous protein

is inserted in the virion, the virion is preferably tested for its ability to cause symptoms in an appropriate animal model since the virus may have acquired new, possibly pathological, properties.

5.4 Adjuvants and Carrier Molecules

[0157] hEbola-associated antigens are administered with one or more adjuvants. In one embodiment, the hEbola-associated antigen is administered together with a mineral salt adjuvants or mineral salt gel adjuvant. Such mineral salt and mineral salt gel adjuvants include, but are not limited to, aluminum hydroxide (ALHYDROGEL, REHYDRAGEL), aluminum phosphate gel, aluminum hydroxyphosphate (ADJU-PHOS), and calcium phosphate.

[0158] In another embodiment, hEbola-associated antigen is administered with an immunostimulatory adjuvant. Such class of adjuvants include, but are not limited to, cytokines (e.g., interleukin-2, interleukin-7, interleukin-12, granulocyte-macrophage colony stimulating factor (GM-CSF), interferon- γ interleukin-1 β (IL-1 β), and IL-1 β peptide or Sclavo Peptide), cytokine-containing liposomes, triterpenoid glycosides or saponins (e.g., QuilA and QS-21, also sold under the trademark STIMULON, ISCOPREP), Muramyl Dipeptide (MDP) derivatives, such as N-acetylmuramyl-L-threonyl-D-isoglutamine (Threonyl-MDP, sold under the trademark TERMURTIDE), GMDP, N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine, N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-s- n-glycero-3-hydroxy phosphoryloxy)-ethylamine, muramyl tripeptide phosphatidylethanolamine (MTP-PE), unmethylated CpG dinucleotides and oligonucleotides, such as bacterial DNA and fragments thereof, LPS, monophosphoryl Lipid A (3D-MLA sold under the trademark MPL), and polyphosphazenes.

[0159] In another embodiment, the adjuvant used is a particular adjuvant, including, but not limited to, emulsions, e.g., Freund's Complete Adjuvant, Freund's Incomplete Adjuvant, squalene or squalane oil-in-water adjuvant formulations, such as SAF and MF59, e.g., prepared with block-copolymers, such as L-121 (polyoxypropylene/polyoxyethylene) sold under the trademark PLURONIC L-121, Liposomes, Virosomes, cochleates, and immune stimulating complex, which is sold under the trademark ISCOM.

[0160] In another embodiment, a microparticulate adjuvant is used. Microparticulate adjuvants include, but are not limited to, biodegradable and biocompatible polyesters, homo- and copolymers of lactic acid (PLA) and glycolic acid (PGA), poly(lactide-co-glycolides) (PLGA) microparticles, polymers that self-associate into particulates (poloxamer particles), soluble polymers (polyphosphazenes), and virus-like particles (VLPs) such as recombinant protein particulates, e.g., hepatitis B surface antigen (HbsAg).

[0161] Yet another class of adjuvants that are optionally used include mucosal adjuvants, including but not limited to heat-labile enterotoxin from *Escherichia coli* (LT), cholera holotoxin (CT) and cholera Toxin B Subunit (CTB) from *Vibrio cholerae*, mutant toxins (e.g., LTK63 and LTR72), microparticles, and polymerized liposomes.

5 **[0162]** In other embodiments, any of the above classes of adjuvants are optionally used in combination with each other or with other adjuvants. For example, non-limiting examples of combination adjuvant preparations used to administer the hEbola-associated antigens of the invention include liposomes containing immunostimulatory protein, cytokines, T-cell and/or B-cell peptides, or microbes with or without entrapped IL-2 or microparticles containing enterotoxin.
10 Other adjuvants known in the art are also included within the scope of the invention (see *Vaccine Design: The Subunit and Adjuvant Approach*, Chap. 7, Michael F. Powell and Mark J. Newman (eds.), Plenum Press, New York, 1995, which is incorporated herein in its entirety).

[0163] The effectiveness of an adjuvant is illustratively determined by measuring the induction of antibodies directed against an immunogenic polypeptide containing a hEbola polypeptide epitope,
15 the antibodies resulting from administration of this polypeptide in vaccines which are also comprised of the various adjuvants.

[0164] The polypeptides are optionally formulated into the vaccine as neutral or salt forms. Pharmaceutically acceptable salts include the acid additional salts (formed with free amino groups of the peptide) and which are formed with inorganic acids, such as, for example, hydrochloric or
20 phosphoric acids, or organic acids such as acetic, oxalic, tartaric, maleic, and the like. Salts formed with free carboxyl groups are optionally derived from inorganic bases, such as, for example, sodium potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine and the like.

[0165] The vaccines of the invention are preferably multivalent or univalent. Multivalent
25 vaccines are made from recombinant viruses that direct the expression of more than one antigen.

[0166] Many methods are operable herein to introduce the vaccine formulations of the invention; these include but are not limited to oral, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal routes, and via scarification (scratching through the top layers of skin, e.g., using a bifurcated needle).

30 **[0167]** The patient to which the vaccine is administered is preferably a mammal, most preferably a human, but is also optionally a non-human animal including but not limited to lower primates, cows, horses, sheep, pigs, fowl (e.g., chickens), goats, cats, dogs, hamsters, mice and rats.

Preparation of Antibodies

[0168] Antibodies that specifically recognize a polypeptide of the invention, such as, but not limited to, polypeptides including the sequence of SEQ ID NOs: 2-9, 59, or 11-19 and other polypeptides as described herein, or hEbola epitope or antigen-binding fragments thereof are used in a preferred embodiment for detecting, screening, and isolating the polypeptide of the invention or fragments thereof, or similar sequences that might encode similar enzymes from the other organisms. For example, in one specific embodiment, an antibody which immunospecifically binds hEbola epitope, or a fragment thereof, is used for various *in vitro* detection assays, including enzyme-linked immunosorbent assays (ELISA), radioimmunoassays, western blot, etc., for the detection of a polypeptide of the invention or, preferably, hEbola, in samples, for example, a biological material, including cells, cell culture media (e.g., bacterial cell culture media, mammalian cell culture media, insect cell culture media, yeast cell culture media, etc.), blood, plasma, serum, tissues, sputum, nasopharyngeal aspirates, etc.

[0169] Antibodies specific for a polypeptide of the invention or any epitope of hEbola are optionally generated by any suitable method known in the art. Polyclonal antibodies to an antigen of interest, for example, the hEbola virus from Deposit Accession No. 200706291, or including a nucleotide sequence of SEQ ID NOs: 1 or 10, are optionally produced by various procedures well known in the art. For example, an antigen is optionally administered to various host animals including, but not limited to, rabbits, mice, rats, etc., to induce the production of antisera containing polyclonal antibodies specific for the antigen. Various adjuvants are optionally used to increase the immunological response, depending on the host species, and include but are not limited to, Freund's (complete and incomplete) adjuvant, mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful adjuvants for humans such as BCG (Bacille Calmette-Guerin) and *Corynebacterium parvum*. Such adjuvants are also well known in the art.

[0170] Monoclonal antibodies are optionally prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. In one example, monoclonal antibodies are produced using hybridoma techniques including those known in the art and taught, for example, in Harlow et al., *Antibodies: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling, et al., in: *Monoclonal Antibodies and T-Cell Hybridomas*, pp. 563-681 (Elsevier, N.Y., 1981) (both of which

are incorporated by reference in their entireties). The term “monoclonal antibody” as used herein is not limited to antibodies produced through hybridoma technology. The term “monoclonal antibody” refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

5 [0171] Methods for producing and screening for specific antibodies using hybridoma technology are routine and well known in the art. In a non-limiting example, mice are immunized with an antigen of interest or a cell expressing such an antigen. Once an immune response is detected, e.g., antibodies specific for the antigen are detected in the mouse serum, the mouse spleen is harvested and splenocytes isolated. The splenocytes are then fused by well known techniques to
10 any suitable myeloma cells. Hybridomas are selected and cloned by limiting dilution. The hybridoma clones are then assayed by methods known in the art for cells that secrete antibodies capable of binding the antigen. Ascites fluid, which generally contains high levels of antibodies, is optionally generated by inoculating mice intraperitoneally with positive hybridoma clones.

[0172] Antibody fragments which recognize specific epitopes are optionally generated by
15 known techniques. For example, Fab and F(ab')₂ fragments are illustratively produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')₂ fragments). F(ab')₂ fragments preferably contain the complete light chain, and the variable region, the CH1 region and the hinge region of the heavy chain.

[0173] The antibodies of the invention or fragments thereof are optionally produced by any
20 method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or preferably, by recombinant expression techniques.

[0174] The nucleotide sequence encoding an antibody is obtained from any information available to those skilled in the art (i.e., from Genbank, the literature, or by routine cloning and sequence analysis). If a clone containing a nucleic acid encoding a particular antibody or an epitope-
25 binding fragment thereof is not available, but the sequence of the antibody molecule or epitope-binding fragment thereof is known, a nucleic acid encoding the immunoglobulin may be chemically synthesized or obtained from a suitable source (e.g., an antibody cDNA library, or a cDNA library generated from, or nucleic acid, preferably poly A+RNA, isolated from any tissue or cells expressing the antibody, such as hybridoma cells selected to express an antibody) by PCR amplification using
30 synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, e.g., a cDNA clone from

a cDNA library that encodes the antibody. Amplified nucleic acids generated by PCR are optionally then cloned into replicable cloning vectors using any method known in the art.

[0175] Once the nucleotide sequence of the antibody is determined, the nucleotide sequence of the antibody is optionally manipulated using methods well known in the art for the manipulation of nucleotide sequences, e.g., recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, 5 for example, the techniques described in Sambrook et al., *supra*; and Ausubel et al., eds., 1998, Current Protocols in Molecular Biology, John Wiley & Sons, NY, which are both incorporated by reference herein in their entireties), to generate antibodies having a different amino acid sequence by, for example, introducing amino acid substitutions, deletions, and/or insertions into the epitope- 10 binding domain regions of the antibodies or any portion of antibodies which may enhance or reduce biological activities of the antibodies.

[0176] Recombinant expression of an antibody requires construction of an expression vector containing a nucleotide sequence that encodes the antibody. Once a nucleotide sequence encoding an antibody molecule or a heavy or light chain of an antibody, or portion thereof has been obtained, 15 the vector for the production of the antibody molecule is optionally produced by recombinant DNA technology using techniques known in the art as discussed in the previous sections. Methods which are known to those skilled in the art are optionally used to construct expression vectors containing antibody coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, *in vitro* recombinant DNA techniques, synthetic techniques, and *in* 20 *vivo* genetic recombination. The nucleotide sequence encoding the heavy-chain variable region, light-chain variable region, both the heavy-chain and light-chain variable regions, an epitope-binding fragment of the heavy- and/or light-chain variable region, or one or more complementarity determining regions (CDRs) of an antibody are optionally cloned into such a vector for expression. Thus, prepared expression vector is optionally then introduced into appropriate host cells for the 25 expression of the antibody. Accordingly, the invention includes host cells containing a polynucleotide encoding an antibody specific for the polypeptides of the invention or fragments thereof.

[0177] The host cell is optionally co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light 30 chain derived polypeptide. The two vectors illustratively contain identical selectable markers which enable equal expression of heavy and light chain polypeptides or different selectable markers to ensure maintenance of both plasmids. Alternatively, a single vector is optionally used which

encodes, and is capable of expressing, both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, *Nature*, 322:52, 1986; and Kohler, *Proc. Natl. Acad. Sci. USA*, 77:2 197, 1980). The coding sequences for the heavy and light chains optionally include cDNA or genomic DNA.

5 **[0178]** In another embodiment, antibodies are generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In a particular embodiment, such phage is utilized to display antigen binding domains, such as Fab and Fv or disulfide-bond stabilized Fv, expressed from a repertoire or combinatorial antibody library (e.g.,
10 human or murine). Phage expressing an antigen binding domain that binds the antigen of interest is optionally selected or identified with antigen, e.g., using labeled antigen or antigen bound or captured to a solid surface or bead. Phages used in these methods are typically filamentous phage, including fd and M13. The antigen binding domains are expressed as a recombinantly fused protein to either the phage gene III or gene VIII protein. Examples of phage display methods that can be
15 used to make the immunoglobulins, or fragments thereof, of the present invention include those disclosed in Brinkman et al., *J. Immunol. Methods*, 182:41-50, 1995; Ames et al., *J. Immunol. Methods*, 184:177-186, 1995; Kettleborough et al., *Eur. J. Immunol.*, 24:952-958, 1994; Persic et al., *Gene*, 187:9-18, 1997; Burton et al., *Advances in Immunology*, 57:191-280, 1994; PCT application No. PCT/GB91/01134; PCT publications WO 90/02809; WO 91/10737; WO 92/01047; WO
20 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Pat. Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

[0179] As described in the above references, after phage selection, the antibody coding regions
25 from the phage is optionally isolated and used to generate whole antibodies, including human antibodies, or any other desired fragments, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described in detail below. For example, techniques to recombinantly produce Fab, Fab' and F(ab')₂ fragments are optionally employed using methods known in the art such as those disclosed in PCT publication WO 92/22324; Mullinax et al.,
30 *BioTechniques*, 12(6):864-869, 1992; and Sawai et al., *AJRI*, 34:26-34, 1995; and Better et al., *Science*, 240:1041-1043, 1988 (each of which is incorporated by reference in its entirety). Examples of techniques operable to produce single-chain Fvs and antibodies include those described in U.S.

Pat. Nos. 4,946,778 and 5,258,498; Huston et al., *Methods in Enzymology*, 203:46-88, 1991; Shu et al., *PNAS*, 90:7995-7999, 1993; and Skerra et al., *Science*, 240:1038-1040, 1988.

[0180] Once an antibody molecule of the invention has been produced by any methods described above, or otherwise known in the art, it is then optionally purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigen after Protein A or Protein G purification, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique(s) for the purification of proteins. Further, the antibodies of the present invention or fragments thereof are optionally fused to heterologous polypeptide sequences described herein or otherwise known in the art to facilitate purification. Illustrative examples include 6xHis tag, FLAG tag, biotin, avidin, or other system.

[0181] For some uses, including *in vivo* use of antibodies in humans and *in vitro* detection assays, it is preferable to use chimeric, humanized, or human antibodies. A chimeric antibody is a molecule in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal antibody and a constant region derived from a human immunoglobulin. Methods for producing chimeric antibodies are known in the art. See e.g., Morrison, *Science*, 229:1202, 1985; Oi et al., *BioTechniques*, 4:214-218, 1986; Gillies et al., *J. Immunol. Methods*, 125:191-202, 1989; U.S. Pat. Nos. 5,807,715; 4,816,567; and 4,816,397, which are incorporated herein by reference in their entireties. Humanized antibodies are antibody molecules from non-human species that bind the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and framework regions from a human immunoglobulin molecule. Often, framework residues in the human framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. See, e.g., Queen et al., U.S. Pat. No. 5,585,089; Riechmann et al., *Nature*, 332:323, 1988, which are incorporated herein by reference in their entireties. Antibodies are humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; PCT publication WO 91/09967; U.S. Pat. Nos. 5,225,539; 5,530,101 and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, *Molecular Immunology*, 28(4/5):489-498, 1991; Studnicka et al., *Protein Engineering*, 7(6):805-814, 1994;

Roguska et al., Proc Natl. Acad. Sci. USA, 91:969-973, 1994), and chain shuffling (U.S. Pat. No. 5,565,332), all of which are hereby incorporated by reference in their entireties.

[0182] Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Human antibodies are made by a variety of methods known in the art illustratively including phage display methods described above using antibody libraries derived from human immunoglobulin sequences. See U.S. Pat. Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645; WO 98/50433; WO 98/24893; WO 98/16654; WO 96/34096; WO 96/33735; and WO 91/10741, each of which is incorporated herein by reference in its entirety.

[0183] Human antibodies are also illustratively produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. For an overview of this technology for producing human antibodies, see Lonberg and Huszar, Int. Rev. Immunol., 13:65-93, 1995. For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, e.g., PCT publications WO 98/24893; WO 92/01047; WO 96/34096; WO 96/33735; European Patent No. 0 598 877; U.S. Pat. Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; 5,885,793; 5,916,771; and 5,939,598, which are incorporated by reference herein in their entireties. In addition, companies such as Abgenix, Inc. (Fremont, Calif.), Medarex (NJ) and Genpharm (San Jose, Calif.) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

[0184] Completely human antibodies which recognize a selected epitope are optionally generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, e.g., a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespers et al., Bio/technology, 12:899-903, 1988).

[0185] Antibodies fused or conjugated to heterologous polypeptides are optionally used in *in vitro* immunoassays and in purification methods (e.g., affinity chromatography) known in the art. See e.g., PCT publication No. WO 93/21232; EP 439,095; Naramura et al., Immunol. Lett., 39:91-99, 1994; U.S. Pat. No. 5,474,981; Gillies et al., PNAS, 89:1428-1432, 1992; and Fell et al., J. Immunol., 146:2446-2452, 1991, which are incorporated herein by reference in their entireties.

[0186] Antibodies may also be illustratively attached to solid supports, which are particularly useful for immunoassays or purification of the polypeptides of the invention or fragments, derivatives, analogs, or variants thereof, or similar molecules having the similar enzymatic activities

as the polypeptide of the invention. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

Pharmaceutical Compositions and Kits

5 **[0187]** The present invention encompasses pharmaceutical compositions including antiviral agents of the present invention. In a specific embodiment, the antiviral agent is preferably an antibody which immunospecifically binds and neutralizes the hEbola virus or variants thereof, or any proteins derived therefrom. In another specific embodiment, the antiviral agent is a polypeptide or nucleic acid molecule of the invention. The pharmaceutical compositions have utility as an
10 antiviral prophylactic agent are illustratively administered to a subject where the subject has been exposed or is expected to be exposed to a virus.

[0188] Various delivery systems are known and operable to administer the pharmaceutical composition of the invention, illustratively, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the mutant viruses, and receptor mediated
15 endocytosis (see, e.g., Wu and Wu, 1987, J. Biol. Chem. 262:4429-4432). Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous
20 linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and optionally administered together with other biologically active agents. Administration is systemic or local. In a preferred embodiment, it is desirable to introduce the pharmaceutical compositions of the invention into the lungs by any suitable route. Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

[0189] In a specific embodiment, it is desirable to administer the pharmaceutical compositions
25 of the invention locally to the area in need of treatment. This administration may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, e.g., in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, by means of nasal spray, or by means of an implant, the implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. In one
30 embodiment, administration can be by direct injection at the site (or former site) infected tissues.

[0190] In another embodiment, the pharmaceutical composition is delivered in a vesicle, in particular a liposome (see Langer, 1990, Science 249:1527-1533; Treat et al., in Liposomes in the

Therapy of Infectious Disease and Cancer, Lopez Berestein and Fidler (eds.), Liss, New York, pp. 353-365 (1989); Lopez-Berestein, *ibid.*, pp. 317-327; see generally *ibid.*).

[0191] In yet another embodiment, the pharmaceutical composition is delivered in a controlled release system. In one embodiment, a pump is used (see Langer, *supra*; Sefton, 1987, CRC Crit. Ref. Biomed. Eng. 14:201; Buchwald et al., 1980, Surgery 88:507; and Saudek et al., 1989, N. Engl. J. Med. 321:574). In another embodiment, polymeric materials are used (see Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Fla. (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J. Macromol. Sci. Rev. Macromol. Chem. 23:61 (1983); see also Levy et al., 1985, Science 228:190; During et al., 1989, Ann. Neurol. 25:351; Howard et al., 1989, J. Neurosurg. 71:105). In yet another embodiment, a controlled release system is placed in proximity of the composition's target, i.e., the lung, thus, requiring only a fraction of the systemic dose (see, e.g., Goodson, in Medical Applications of Controlled Release, *supra*, vol. 2, pp. 115-138 (1984)).

[0192] Other controlled release systems are discussed in the review by Langer (Science 249:1527-1533 (1990)) the contents of which are incorporated herein by reference.

[0193] The pharmaceutical compositions of the present invention illustratively include a therapeutically effective amount of a live attenuated, inactivated or killed West African hEbola virus, or recombinant or chimeric hEbola virus, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the pharmaceutical composition is administered. Such pharmaceutical carriers are illustratively sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions are optionally employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, also contains wetting or emulsifying agents, or pH buffering agents. These compositions optionally take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained release formulations and

the like. The composition is optionally formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation illustratively includes standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in 5 “Remington’s Pharmaceutical Sciences” by E. W. Martin. The formulation should suit the mode of administration.

[0194] In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic 10 aqueous buffer. The composition also includes an optional solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water-free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be 15 dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline is optionally provided so that the ingredients may be mixed prior to administration.

[0195] The pharmaceutical compositions of the invention are illustratively formulated as neutral or salt forms. Pharmaceutically acceptable salts illustratively include those formed with free amino 20 groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2 ethylamino ethanol, histidine, procaine, etc.

[0196] The amount of the pharmaceutical composition of the invention which will be effective 25 in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. In addition, *in vitro* assays are optionally employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient’s 30 circumstances. However, suitable dosage ranges for intravenous administration are generally about 20 to 500 micrograms of active compound per kilogram body weight. Suitable dosage ranges for intranasal administration are generally about 0.01 pg/kg body weight to 1 mg/kg body weight.

Effective doses may be extrapolated from dose response curves derived from *in vitro* or animal model test systems.

[0197] Suppositories generally contain active ingredient in the range of 0.5% to 10% by weight; oral formulations preferably contain 10% to 95% active ingredient.

5 **[0198]** The invention also provides a pharmaceutical pack or kit including one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) is a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human
10 administration. In a preferred embodiment, the kit contains an antiviral agent of the invention, e.g., an antibody specific for the polypeptides encoded by a nucleotide sequence of SEQ ID NOs: 1 or 10, or as shown in SEQ ID NOs: 2-9, 59, or 11-19, or any hEbola epitope, or a polypeptide or protein of the present invention, or a nucleic acid molecule of the invention, alone or in combination with adjuvants, antivirals, antibiotics, analgesic, bronchodilators, or other pharmaceutically acceptable
15 excipients.

[0199] The present invention further encompasses kits including a container containing a pharmaceutical composition of the present invention and instructions for use.

Detection Assays

20 **[0200]** The present invention provides a method for detecting an antibody, which immunospecifically binds to the hEbola virus, in a biological sample, including for example blood, serum, plasma, saliva, urine, feces, etc., from a patient suffering from hEbola infection, and/or hemorrhagic fever. In a specific embodiment, the method including contacting the sample with the hEbola virus, for example, of Deposit Accession No. 200706291, or having a genomic nucleic acid
25 sequence of SEQ ID NOs: 1 or 10, directly immobilized on a substrate and detecting the virus-bound antibody directly or indirectly by a labeled heterologous anti-isotype antibody. In another specific embodiment, the sample is contacted with a host cell which is infected by the hEbola virus, for example, of Deposit Accession No. 200706291, or having a genomic nucleic acid sequence of SEQ ID NOs: 1 or 10, and the bound antibody is optionally detected by immunofluorescent assay.

30 **[0201]** An exemplary method for detecting the presence or absence of a polypeptide or nucleic acid of the invention in a biological sample involves obtaining a biological sample from various sources and contacting the sample with a compound or an agent capable of detecting an epitope or

nucleic acid (e.g., mRNA, genomic DNA) of the hEbola virus such that the presence of the hEbola virus is detected in the sample. A preferred agent for detecting hEbola mRNA or genomic RNA of the invention is a labeled nucleic acid probe capable of hybridizing to mRNA or genomic RNA encoding a polypeptide of the invention. The nucleic acid probe is, for example, a nucleic acid molecule including the nucleotide sequence of SEQ ID NOs: 1 or 10, a complement thereof, or a portion thereof, such as an oligonucleotide of at least 15, 20, 25, 30, 50, 100, 250, 500, 750, 1000 or more contiguous nucleotides in length and sufficient to specifically hybridize under stringent conditions to a hEbola mRNA or genomic RNA.

[0202] As used herein, the term “stringent conditions” describes conditions for hybridization and washing under which nucleotide sequences having at least 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% identity to each other typically remain hybridized to each other. Such hybridization conditions are described in, for example but not limited to, Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1 6.3.6.; Basic Methods in Molecular Biology, Elsevier Science Publishing Co., Inc., N.Y. (1986), pp.75 78, and 84 87; and Molecular Cloning, Cold Spring Harbor Laboratory, N.Y. (1982), pp.387 389, and are well known to those skilled in the art. A preferred, non-limiting example of stringent hybridization conditions is hybridization in 6x sodium chloride/sodium citrate (SSC), 0.5% SDS at about 68 °C followed by one or more washes in 2xSSC, 0.5% SDS at room temperature. Another preferred, non-limiting example of stringent hybridization conditions is hybridization in 6x SSC at about 45°C followed by one or more washes in 0.2x SSC, 0.1% SDS at 50 to 65 °C.

[0203] A nucleic acid probe, polynucleotide, oligonucleotide, or other nucleic acid is preferably purified. An “isolated” or “purified” nucleotide sequence is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the nucleotide is derived, or is substantially free of chemical precursors or other chemicals when chemically synthesized. The language “substantially free of cellular material” includes preparations of a nucleotide/oligonucleotide in which the nucleotide/oligonucleotide is separated from cellular components of the cells from which it is isolated or produced. Thus, a nucleotide/oligonucleotide that is substantially free of cellular material includes preparations of the nucleotide having less than about 30%, 20%, 10%, 5%, 2.5%, or 1%, (by dry weight) of contaminating material. When nucleotide/oligonucleotide is produced by chemical synthesis, it is preferably substantially free of chemical precursors or other chemicals, i.e., it is separated from chemical precursors or other chemicals which are involved in the synthesis of the protein. Accordingly, such preparations of the

nucleotide/oligonucleotide have less than about 30%, 20%, 10%, or 5% (by dry weight) of chemical precursors or compounds other than the nucleotide/oligonucleotide of interest. In a preferred embodiment of the present invention, the nucleotide/oligonucleotide is isolated or purified.

[0204] In another preferred specific embodiment, the presence of hEbola virus is detected in the sample by a reverse transcription polymerase chain reaction (RT-PCR) using the primers that are constructed based on a partial nucleotide sequence of the genome of hEbola virus, for example, that of Deposit Accession No. 200706291, or having a genomic nucleic acid sequence of SEQ ID NOs: 1 or 10. In a non-limiting specific embodiment, preferred primers to be used in a RT-PCR method are the primers are described in detail herein.

[0205] In more preferred specific embodiment, the present invention provides a real-time quantitative PCR assay to detect the presence of hEbola virus in a biological sample by subjecting the cDNA obtained by reverse transcription of the extracted total RNA from the sample to PCR reactions using the specific primers described in detail herein, and a fluorescence dye, such as SYBR® Green I, which fluoresces when bound nonspecifically to double-stranded DNA. The fluorescence signals from these reactions are captured at the end of extension steps as PCR product is generated over a range of the thermal cycles, thereby allowing the quantitative determination of the viral load in the sample based on an amplification plot.

[0206] A preferred agent for detecting hEbola is an antibody that specifically binds a polypeptide of the invention or any hEbola epitope, preferably an antibody with a detectable label. Antibodies are illustratively polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (e.g., Fab or F(ab')₂) is operable herein.

[0207] The term “labeled”, with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (i.e., physically linking) a detectable substance to the probe or antibody, optionally via a linker, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it is detectable with fluorescently labeled streptavidin. The detection method of the invention is optionally used to detect mRNA, protein (or any epitope), or genomic RNA in a sample *in vitro* as well as *in vivo*. Exemplary *in vitro* techniques for detection of mRNA include northern hybridizations, *in situ* hybridizations, RT-PCR, and RNase protection. *In vitro* techniques for detection of an epitope of hEbola illustratively include enzyme linked immunosorbent assays (ELISAs), western blots, immunoprecipitations and immunofluorescence. *In*

vitro techniques for detection of genomic RNA include northern hybridizations, RT-PCT, and RNase protection. Furthermore, *in vivo* techniques for detection of hEbola include introducing into a subject organism a labeled antibody directed against the polypeptide. In one embodiment, the antibody is labeled with a radioactive marker whose presence and location in the subject organism is
5 detected by standard imaging techniques, including autoradiography.

[0208] In a specific embodiment, the methods further involve obtaining a control sample from a control subject, contacting the control sample with a compound or agent capable of detecting hEbola, e.g., a polypeptide of the invention or mRNA or genomic RNA encoding a polypeptide of the invention, such that the presence of hEbola or the polypeptide or mRNA or genomic RNA
10 encoding the polypeptide is detected in the sample, and comparing the absence of hEbola or the polypeptide or mRNA or genomic RNA encoding the polypeptide in the control sample with the presence of hEbola, or the polypeptide or mRNA or genomic DNA encoding the polypeptide in the test sample.

[0209] The invention also encompasses kits for detecting the presence of hEbola or a
15 polypeptide or nucleic acid of the invention in a test sample. The kit illustratively includes a labeled compound or agent capable of detecting hEbola or the polypeptide or a nucleic acid molecule encoding the polypeptide in a test sample and, in certain embodiments, a means for determining the amount of the polypeptide or mRNA in the sample (e.g., an antibody which binds the polypeptide or an oligonucleotide probe which binds to DNA or mRNA encoding the polypeptide). Kits optionally
20 include instructions for use.

[0210] For antibody-based kits, the kit illustratively includes: (1) a first antibody (e.g., attached to a solid support) which binds to a polypeptide of the invention or hEbola epitope; and, optionally, (2) a second, different antibody which binds to either the polypeptide or the first antibody and is preferably conjugated to a detectable agent.

[0211] For oligonucleotide-based kits, the kit illustratively includes: (1) an oligonucleotide, e.g.,
25 a detectably labeled oligonucleotide, which hybridizes to a nucleic acid sequence encoding a polypeptide of the invention or to a sequence within the hEbola genome; or (2) a pair of primers useful for amplifying a nucleic acid molecule containing an hEbola sequence. The kit optionally includes a buffering agent, a preservative, or a protein stabilizing agent. The kit optionally includes
30 components necessary for detecting the detectable agent (e.g., an enzyme or a substrate). The kit optionally contains a control sample or a series of control samples which can be assayed and compared to the test sample contained. Each component of the kit is usually enclosed within an

individual container and all of the various containers are within a single package along with instructions for use.

Screening Assays to Identify Antiviral Agents

5 [0212] The invention provides methods for the identification of a compound that inhibits the ability of hEbola virus to infect a host or a host cell. In certain embodiments, the invention provides methods for the identification of a compound that reduces the ability of hEbola virus to replicate in a host or a host cell. Any technique well known to the skilled artisan is illustratively used to screen for a compound useful to abolish or reduce the ability of hEbola virus to infect a host and/or to
10 replicate in a host or a host cell.

[0213] In certain embodiments, the invention provides methods for the identification of a compound that inhibits the ability of hEbola virus to replicate in a mammal or a mammalian cell. More specifically, the invention provides methods for the identification of a compound that inhibits the ability of hEbola virus to infect a mammal or a mammalian cell. In certain embodiments, the
15 invention provides methods for the identification of a compound that inhibits the ability of hEbola virus to replicate in a mammalian cell. In a specific embodiment, the mammalian cell is a human cell.

[0214] In another embodiment, a cell is contacted with a test compound and infected with the hEbola virus. In certain embodiments, a control culture is infected with the hEbola virus in the
20 absence of a test compound. The cell is optionally contacted with a test compound before, concurrently with, or subsequent to the infection with the hEbola virus. In a specific embodiment, the cell is a mammalian cell. In an even more specific embodiment, the cell is a human cell. In certain embodiments, the cell is incubated with the test compound for at least 1 minute, at least 5 minutes, at least 15 minutes, at least 30 minutes, at least 1 hour, at least 2 hours, at least 5 hours, at
25 least 12 hours, or at least 1 day. The titer of the virus is optionally measured at any time during the assay. In certain embodiments, a time course of viral growth in the culture is determined. If the viral growth is inhibited or reduced in the presence of the test compound, the test compound is identified as being effective in inhibiting or reducing the growth or infection of the hEbola virus. In a specific embodiment, the compound that inhibits or reduces the growth of the hEbola virus is
30 tested for its ability to inhibit or reduce the growth rate of other viruses to test its specificity for the hEbola virus.

[0215] In one embodiment, a test compound is administered to a model animal and the model animal is infected with the hEbola virus. In certain embodiments, a control model animal is infected with the hEbola virus without the administration of a test compound. The test compound is optionally administered before, concurrently with, or subsequent to the infection with the hEbola virus. In a specific embodiment, the model animal is a mammal. In an even more specific embodiment, the model animal is, but is not limited to, a cotton rat, a mouse, or a monkey. The titer of the virus in the model animal is optionally measured at any time during the assay. In certain embodiments, a time course of viral growth in the culture is determined. If the viral growth is inhibited or reduced in the presence of the test compound, the test compound is identified as being effective in inhibiting or reducing the growth or infection of the hEbola virus. In a specific embodiment, the compound that inhibits or reduces the growth of the hEbola in the model animal is tested for its ability to inhibit or reduce the growth rate of other viruses to test its specificity for the hEbola virus.

[0216] According to the method of the invention, a human or an animal is optionally treated for EboBun or EboIC, other viral infection or bacterial infection by administering an effective amount of an inventive therapeutic composition. Preferably, a vaccine is administered prophylactically. An "effective amount" is an amount that will induce an immune response in a subject. Illustratively, an effective amount of the compositions of this invention ranges from nanogram/kg to milligram/kg amounts for young children and adults. Equivalent dosages for lighter or heavier body weights can readily be determined. The dose should be adjusted to suit the individual to whom the composition is administered and will vary with age, weight and metabolism of the individual. The exact amount of the composition required will vary from subject to subject, depending on the species, age, weight and general condition of the subject, the particular peptide or polypeptide used, its mode of administration and the like. An appropriate amount can be determined by one of ordinary skill in the art using only routine experimentation given the teachings herein. One skilled in the art will realize that dosages are best optimized by the practicing physician or veterinarian and methods for determining dose amounts and regimens and preparing dosage forms are described, for example, in Remington's Pharmaceutical Sciences, (Martin, E. W., ed., latest edition), Mack Publishing Co., Easton, PA. Preferably, a single administration is operable to induce an immune response.

[0217] Methods involving conventional biological techniques are described herein. Such techniques are generally known in the art and are described in detail in methodology treatises such

as Molecular Cloning: A Laboratory Manual, 2nd ed., vol. 1-3, ed. Sambrook et al., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989; and Current Protocols in Molecular Biology, ed. Ausubel et al., Greene Publishing and Wiley-Interscience, New York, 1992 (with periodic updates). Immunological methods (e.g., preparation of antigen-specific antibodies, immunoprecipitation, and immunoblotting) are described, e.g., in Current Protocols in Immunology, ed. Coligan et al., John Wiley & Sons, New York, 1991; and Methods of Immunological Analysis, ed. Masseyeff et al., John Wiley & Sons, New York, 1992.

[0218] Embodiments of inventive compositions and methods are illustrated in the following detailed examples. These examples are provided for illustrative purposes and are not considered limitations on the scope of inventive compositions and methods.

EXAMPLES

Example 1:

Newly discovered Ebola virus associated with hemorrhagic fever outbreak in Bundibugyo, Uganda

[0219] In late November 2007 HF cases were reported in the townships of Bundibugyo and Kikyoo in Bundibugyo District, Western Uganda (Figure 1A). These samples were assayed as described by Towner, JS, et al., *PLoS Pathog*, 2008 November; 4(11): e1000212, the contents of which are incorporated herein by reference for methods, results, reagents, and all other aspects of the publication. A total of 29 blood samples were initially collected from suspect cases and showed evidence of acute ebolavirus infection in eight specimens using a broadly reactive ebolavirus antigen capture assay known to cross-react with the different ebolavirus species⁷ and an IgM capture assay based on *Zaire ebolavirus* reagents (Table 1). These specimens were negative when initially tested with highly sensitive real-time RT-PCR assays specific for all known *Zaire* and *Sudan ebolaviruses* and marburgviruses. However, further evidence of acute ebolavirus infection was obtained using a traditionally less sensitive (relative to the real-time RT-PCR assays) but more broadly reactive filovirus L gene-specific RT-PCR assay (1 specimen) (Table 1). Sequence analysis of the PCR fragment (400 bp of the virus L gene) revealed the reason for the initial failure of the real-time RT-PCR assays, as the sequence was distinct from that of the 4 known species of ebolavirus, although distantly related to *Cote d'Ivoire ebolavirus*. In total, 9 of 29 specimens showed evidence of ebolavirus infection, and all tests were negative for marburgvirus (data not shown).

[0220] Approximately 70% of the virus genome was rapidly sequenced from total RNA extracted from a patient serum (#200706291) using a newly established metagenomics pyrosequencing method (454 Life Sciences) which involves successive rounds of random DNA amplification⁸. Using the newly derived draft sequence, a real-time RT-PCR assay specific for the NP gene of this virus was quickly developed and evaluated. The assay was shown to have excellent sensitivity (Table 1), finding positive all the initial six samples that tested positive by either virus antigen capture (five specimens) or virus isolation assays (four specimens). The antigen-capture, IgM, IgG and newly designed real-time PCR assays were quickly transferred to the Uganda Virus Research Institute during the course of the outbreak to facilitate rapid identification and isolation of Ebola cases in the affected area for efficient control of the outbreak. The outbreak continued through late December 2007, and resulted in 149 suspected cases and 37 deaths⁹.

[0221] Table 1. Ebolavirus diagnostic results of initial 29 specimens obtained from Bundibugyo District with numerical specimen numbers assigned. RT-PCR refers to results obtained from conventional PCR using the broadly reactive Filo A/B primers¹³. Ag, IgM, and IgG refer to results from ELISA-based assays^{10, 11} with *Zaire ebolavirus* reagents while virus isolation refers to culture attempts on Vero E6 cells¹². Q-RT-PCR refers to results obtained using the optimized *Bundibugyo ebolavirus* specific real-time RT-PCR assay with cycle threshold (Ct) values of positive (Pos) samples indicated in the far right column. * Specimen #200706291 is the clinical sample from which prototype isolate #811250 was obtained.

Table 1

Sample No.	RT-PCR	Ag	IgM	IgG	Virus Isolation	Q- RT-PCR	Ct
200706288	neg	neg	neg	neg	neg	neg	40
200706289	neg	neg	neg	neg	neg	neg	40
200706290	neg	neg	neg	neg	neg	neg	40
200706291*	Pos	Pos	neg	neg	Pos	Pos	23.64
200706292	neg	neg	neg	neg	neg	neg	40
200706293	neg	neg	neg	neg	neg	neg	40
200706294	neg	neg	neg	neg	neg	neg	40
200706295	neg	neg	neg	neg	neg	neg	40
200706296	neg	neg	Pos	Pos	neg	neg	40
200706297	neg	neg	Pos	Pos	neg	neg	40
200706298	neg	Pos	Pos	Pos	neg	Pos	34.83
200706299	neg	neg	Pos	Pos	neg	neg	40
200706300	neg	neg	neg	neg	neg	neg	40
200706301	neg	neg	neg	neg	neg	neg	40
200706302	neg	Pos	Pos	neg	neg	Pos	35.01
200706303	neg	neg	neg	neg	neg	neg	40
200706304	neg	neg	neg	neg	Pos	Pos	38.18
200706305	neg	neg	neg	neg	neg	neg	40
200706306	neg	neg	neg	neg	neg	neg	40
200706307	neg	neg	neg	neg	neg	neg	40
200706320	ND	Pos	neg	neg	Pos	Pos	30.24
200706321	ND	neg	neg	neg	neg	neg	40
200706322	ND	neg	neg	neg	neg	neg	40
200706323	ND	neg	neg	neg	neg	neg	40
200706324	ND	neg	neg	neg	neg	neg	40
200706325	ND	neg	neg	neg	neg	neg	40
200706326	ND	neg	neg	neg	neg	neg	40
200706327	ND	Pos	neg	neg	Pos	Pos	34.41
200706328	ND	neg	neg	neg	neg	neg	40

[0222] The entire genome sequence of this virus was completed using a classic primer walking sequencing approach on RNA. The complete genome of the Eb ebolavirus was not available, so it too was derived by a similar combination of random primed pyrosequencing and primer walking approaches. Acquisition of these sequences allowed for the first time the phylogenetic analysis of the complete genomes of representatives of all known species of Ebola and Marburg viruses. The analysis revealed that the newly discovered virus differed from the four existing ebolavirus species (Figure 1), with approximately 32% nucleotide difference from even the closest relative, EboIC (Table 2). Similar complete genome divergence (35-45%) is seen between the previously characterized ebolavirus species.

[0223] Table 2. Identity matrix based on comparisons of full-length genome sequences of *Zaire ebolaviruses* 1976 (Genbank accession number NC_002549) and 1995 (Genbank accession number AY354458), *Sudan ebolavirus* 2000 (Genbank accession number NC_006432), *Cote d'Ivoire*

ebolavirus 1994 (SEQ ID NO: 10), *Reston ebolavirus* 1989 (Genbank accession number NC_004161), and *Bundibugyo ebolavirus* 2007 (SEQ ID NO: 1).

Table 2

		<u>Zaire '95</u>	<u>Sudan '00</u>	<u>EboIC '94</u>	<u>EboBun '07</u>	<u>Reston '89</u>
5	Zaire '76	.988	.577	.630	.632	.581
	Zaire '95		.577	.631	.633	.581
	Sudan '00			.577	.577	.609
	EboIC '94				.683	.575
	EboBun '07				.576	

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[0224] The material and information obtained from the discovery of the new unique virus EboBun and the realization that together with EboIC these viruses represent a clade of Bundibungyo-Ivory Coast Ebola virus species is valuable, and makes possible the development of clinical, diagnostic and research tools directed to human hEbola infection.

15 **Material and Methods**

[0225] *Ebolavirus detection and virus isolation.* Several diagnostic techniques were used for each sample: (i) antigen capture, IgG, and IgM assays were performed as previously described¹¹ (ii) virus isolation attempts were performed on Vero E6 cells¹² and monitored for 14 days; (iii) RNA was extracted and tested for *Zaire*¹⁶ and *Sudan ebolavirus* and marburgvirus⁴ using real-time quantitative RT-PCR assays designed to detect all known species of each respective virus species the primers/probe for the *Sudan ebolavirus* assay were EboSudBMG 1(+) 5'-GCC ATG GIT TCA GGT TTG AG-3' (SEQ ID NO: 21), EboSudBMG 1(-) 5'-GGT IAC ATT GGG CAA CAA TTC A-3' (SEQ ID NO: 22) and Ebola Sudan BMG Probe 5'FAM-AC GGT GCA CAT TCT CCT TTT CTC GGA-BHQ1 (SEQ ID NO: 23)]; (iv) the conventional RT-PCR was performed with the filo A/B primer set as previously described¹⁶ using Superscript III (Invitrogen) according to the manufacturer's instructions. The specimen 200706291 was selected as the reference sample for further sequence analysis.

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[0226] *Genome sequencing.* Pyrosequencing was carried out utilizing the approach developed by 454 Life Sciences, and the method described by Cox-Foster et al.⁸ Subsequent virus whole genome primer walking was performed as previously described¹⁷ but using the primers specific for *Bundibugyo ebolavirus* RT-PCR amplification. In total, the entire virus genome was amplified in six overlapping RT-PCR fragments (all primers listed 5' to 3'): fragment A (predicted size 2.7 kb)

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was amplified using forward-GTGAGACAAAGAATCATTCTG (SEQ ID NO: 24) with reverse-CATCAATTGCTCAGAGATCCACC (SEQ ID NO: 25); fragment B (predicted size 3.0 kb) was amplified using forward-CCAACAACACTGCATGTAAGT (SEQ ID NO: 26) with reverse-AGGTCGCGTTAATCTTCATC (SEQ ID NO: 27); fragment C (predicted size 3.5 kb) was amplified using forward-GATGGTTGAGTTACTTTCCGG (SEQ ID NO: 28) with reverse-GTCTTGAGTCATCAATGCCC (SEQ ID NO: 29); fragment D (predicted size 3.1 kb) was amplified using forward-CCACCAGCACCAAAGGAC (SEQ ID NO: 30) with reverse-CTATCGGCAATGTAAGTATTGG (SEQ ID NO: 31); fragment E (predicted size 3.4 kb) was amplified using forward-GCCGTTGTAGAGGACACAC (SEQ ID NO: 32) with reverse-CACATTAATTGTTCTAACATGCAAG (SEQ ID NO: 33) and fragment F (predicted size 3.5 kb) was amplified using forward-CCTAGGTTATTTAGAAGGGACTA (SEQ ID NO: 34) with reverse-GGT AGA TGT ATT GAC AGC AAT ATC (SEQ ID NO: 35).

[0227] The exact 5' and 3' ends of *Bundibugyo ebolavirus* were determined by 3' RACE from virus RNA extracted from virus infected Vero E6 cell monolayers using TriPure isolation reagent. RNAs were then polyadenylated *in vitro* using A-Plus poly(A) polymerase tailing kit (Epicenter Biotechnologies) following the manufacturer's instructions and then purified using an RNeasy kit (Qiagen) following standard protocols. Ten microliters of *in vitro* polyadenylated RNA were added as template in RT-PCR reactions, using SuperScript III One-Step RT-PCR system with Platinum *Taq* High Fidelity (Invitrogen) following the manufacturer's protocol. Two parallel RT-PCR reactions using the oligo(dT)-containing 3'RACE-AP primer (Invitrogen) mixed with 1 of 2 viral specific primers, Ebo-U 692(-) ACAAAAAGCTATCTGCACTAT (SEQ ID NO: 36) and Ebo-U18269(+) CTCAGAAGCAAATAATGG (SEQ ID NO: 37), generated ~700 nt long fragments containing the 3' ends of either genomic and antigenomic RNAs. The resulting RT-PCR products were analyzed by agarose electrophoresis, and DNA bands of the correct sizes were purified using QIAquick Gel Extraction Kit (Qiagen) and sequenced using standard protocols (ABI).

[0228] The nucleotide sequence of the *Côte d'Ivoire ebolavirus* (EboIC) isolate RNA was initially determined using the exact same pyrosequencing strategy as that used for *Bundibugyo ebolavirus* described above. This method generated sequence for approximately 70% of the entire genome. This draft sequence was then used to design a whole genome primer walking strategy for filling any gaps and confirming the initial sequence. The following *Côte d'Ivoire ebolavirus*-specific primers were used to generate RT-PCR fragments, designated A-F, as follows: Fragment A (predicted size 3.0 kb) was amplified using forward-GTGTGCGAATAACTATGAGGAAG (SEQ

ID NO: 38) and reverse-GTCTGTGCAATGTTGATGAAGG (SEQ ID NO: 39); Fragment B (predicted size 3.2 kb) was amplified using forward-CATGAAAACCACACTCAACAAC (SEQ ID NO: 40) and reverse-GTTGCCTTAATCTTCATCAAGTTC (SEQ ID NO: 41); Fragment C (predicted size 3.0 kb) was amplified using forward-GGCTATAATGAATTCCTCCAG (SEQ ID NO: 42) and reverse-CAAGTGTATTTGTGGTCCTAGC (SEQ ID NO: 43); fragment D (predicted size 3.5 kb) was amplified using forward-GCTGGAATAGGAATCACAGG (SEQ ID NO: 44) and reverse-CGGTAGTCTACAGTTCTTTAG (SEQ ID NO: 45); fragment E (predicted size 4.0 kb) was amplified using forward-GACAAAGAGATTAGATTAGCTATAG (SEQ ID NO: 46) and reverse-GTAATGAGAAGGTGTCATTTGG (SEQ ID NO: 47); fragment F (predicted size 2.9 kb) was amplified using forward-CACGACTTAGTTGGACAATTGG (SEQ ID NO: 48) and reverse-CAGACACTAATTAGATCTGGAAG (SEQ ID NO: 49); fragment G (predicted size 1.3 kb) was amplified using forward-CGGACACACAAAAAGAAWRAA (SEQ ID NO: 50) and reverse-CGTTCTTGACCTTAGCAGTTC (SEQ ID NO: 51); and fragment H (predicted size 2.5 kb) was amplified using forward-GCACTATAAGCTCGATGAAGTC (SEQ ID NO: 52) and reverse-TGGACACACAAAAARGARAA (SEQ ID NO: 53). A gap in the sequence contig was located between fragments C and D and this was resolved using the following primers to generate a predicted fragment of 1.5 kb: forward-CTGAGAGGATCCAGAAGAAAG (SEQ ID NO: 54) and reverse-GTGTAAGCGTTGATATACCTCC (SEQ ID NO: 55). The terminal ~20 nucleotides of the sequence were not experimentally determined but were inferred by comparing with the other known Ebola genome sequences.

[0229] *Bundibugyo ebolavirus real-time RT-PCR assay.* The primers and probe used in the *Bundibugyo ebolavirus* specific Q-RT-PCR assay were as follows: EboU965(+): 5'-GAGAAAAGGCCTGTCTGGAGAA-3' (SEQ ID NO: 56), EboU1039(-): 5'-TCGGGTATTGAATCAGACCTTGTT-3' (SEQ ID NO: 57) and EboU989 Prb: 5'-Fam-TTCAACGACAAATCCAAGTGCACGCA-3' BHQ1 (SEQ ID NO 58). Q-RT-PCR reactions were set up using Superscript III One-Step Q-RT-PCR (Invitrogen) according to the manufacturer's instructions and run for 40 cycles with a 58°C annealing temperature.

[0230] *Phylogenetic analysis.* Modeltest 3.7¹⁸ was used to examine 56 models of nucleotide substitution to determine the model most appropriate for the data. The General Time Reversible model incorporating invariant sites and a gamma distribution (GTR+I+G) was selected using the Akaike Information Criterion (AIC). Nucleotide frequencies were A = 0.3278, C = 0.2101, G = 0.1832, T = 0.2789, the proportion of invariant sites = 0.1412, and the gamma shape parameter =

1.0593. A maximum likelihood analysis was subsequently performed in PAUP*4.0b10¹⁹ using the GTR+I+G model parameters. Bootstrap support values were used to assess topological support and were calculated based on 1,000 pseudoreplicates²⁰.

[0231] In addition, a Bayesian phylogenetic analysis was conducted in MrBayes 3.2²¹ using the
5 GTR+I+G model of nucleotide substitution. Two simultaneous analyses, each with four Markov chains, were run for 5,000,000 generations sampling every 100 generations. Prior to termination of the run, the AWTY module was used to assess Markov Chain Monte Carlo convergence to ensure that the length of the analysis was sufficient²². Trees generated before the stabilization of the likelihood scores were discarded (burn in = 40), and the remaining trees were used to construct a
10 consensus tree. Nodal support was assessed by posterior probability values (≥ 95 = statistical support).

Example 2

[0232] Immunization against EboBun:

15 [0233] To determine the capability of immunogens to elicit an immune response in non-human primates (NHP), 12 cynomolgus macaques, of which 10 are immunized with VSV Δ G/EboBunGP either orally (OR; n = 4), intranasally (IN; n = 4) or intramuscularly (IM; n = 2) in accordance with all animal control and safety guidelines and essentially as described by Qiu, X, et al., PLoS ONE. 2009; 4(5): e5547. The remaining 2 control animals are vaccinated intramuscularly with
20 VSV Δ G/MARVGP. VSV Δ G/MARVGP does not provide heterologous protection against EboBun, therefore these NHPs succumb to EboBun infection. Animals are acclimatized for 14 days prior to infection. Animals are fed and monitored twice daily (pre- and post-infection) and fed commercial monkey chow, treats and fruit. Husbandry enrichment consists of commercial toys and visual stimulation.

25 [0234] The recombinant VSV Δ G/EboBun vaccines are synthesized expressing the EboBun glycoprotein (GP) (SEQ ID NO: 9), soluble glycoprotein (sGP) (SEQ ID NO: 4), or nucleoprotein (NP) (SEQ ID NO: 3). Control VSV Δ G/MARVGP vaccines represent the analogous proteins from Lake victoria marburgvirus (MARV) (strain Musoke). The following results for GP are similar for sGP and NP. Vaccines are generated using VSV (Indiana serotype) as described previously.
30 Garbutt, M, et al., *J Virol*, 2004; 78(10):5458–5465; Schnell, MJ, et al., *PNAS USA*, 1996; 93(21):11359–11365. EboBun challenge virus is passaged in Vero E6 cells prior to challenge, as described previously Jones, SM, et al., *Nat Med*, 2005; 11(7):786–790; Jahrling, PB, et al., *J Infect*

Dis, 1999; 179(Suppl 1):S224–34. An EboBun immunogen peptide pool consisting of 15mers with 11 amino acid overlaps (Sigma-Genosys) spanning the entire sequence of the EboBun immunogens and strain Mayinga 1976 GP are used.

5 [0235] Twelve filovirus naïve cynomolgus monkeys randomized into four groups receive 2 ml of 1×10^7 PFU/ml of vaccine in Dulbecco's modified Eagle's medium (DMEM). Animals in the three experimental groups are vaccinated with either: 1) 2 ml orally (OR) (n = 4); 2) 1 ml dripped into each nostril, intranasally (IN) (n = 4); or 3) 1 ml each into two sites intramuscularly (IM) (n = 2). The two controls are injected intramuscularly with 2 ml of 1×10^7 PFU/ml of VSV Δ G/MARVGP. All animals are challenged intramuscularly 28 days later with 1,000 PFU of EboBun.

10 [0236] Routine examination is conducted on 0, 2, 4, 6, 10, 14 and 21 days post-vaccination, then 0, 3, 6, 10, 14, 19, 26 days, 6 and 9 months after the EboBun challenge. For the examinations animals are anaesthetized by intramuscular injection with 10 mg/kg of ketaset (Ayerst). Examinations include haematological analysis, monitoring temperature (rectal), respiration rate, lymph nodes, weight, hydration, discharges and mucous membranes. Also, swabs (throat, oral, nasal, 15 rectal, vaginal) and blood samples are collected (4 ml from femoral vein, 1 ml in EDTA vacutainer tube; 3 ml in serum separator vacutainer tube). Cynomolgus monkey PBMCs are isolated using BD CPT sodium citrate Vacutainers (Becton Dickinson) as per manufacturer's protocol.

[0237] All VSV Δ G/EboBunGP immunized animals are protected from high dose challenge. These animals show no evidence of clinical illness after vaccination or EboBun challenge. Both 20 control animals demonstrate typical symptoms associated with EboBun HF including fever, macular rashes, lethargy, and unresponsiveness. Continued infection requires euthanization. Hematology analyses at each examination date demonstrate increases in the platelet-crit in the OR and IN groups post-challenge, however, no significant changes are observed in any NHPs post-immunization or in the VSV Δ G/EboBunGP immunized NHPs post-challenge.

25 [0238] EboBun antibody production from humoral antibody response to vaccination and challenge is examined by a virus like particle (VLP) based ELISA assay. Generation of EboBun VLPs is performed by the protocol for ZEBOV as described by Wahl-Jensen, V., et al., *J Virol*, 2005; 79(4):2413–2419. ELISA is performed by the protocol described by Qiu, X, et al., *PLoS ONE*. 2009; 4(5): e5547.

30 [0239] The VSV Δ G/MARVGP immunized animals do not develop a detectable antibody response to EboBun. In contrast, potent antibody responses are detected in all VSV Δ G/EboBunGP immunized animals independent of immunization route. Between days 14 and 21 post-vaccination,

all VSVΔG/EboBunGP immunized NHPs develop high levels of IgA, IgM, and IgG against EboBunGP. After challenge the IgM titres do not exceed the post-vaccination levels, however, IgG and IgA antibody titres are increased peaking 14 days post-challenge then slowly decreasing before maintaining a relatively high antibody titre up to 9 months.

5 [0240] The level of neutralization antibodies is detected by a EboBun-GFP flow cytometric neutralization assay in serum collected at days 0 and 21 post-vaccination. Samples are assayed in duplicate for their ability to neutralize an infection with EboBun-GFP in VeroE6 cells. Serially diluted serum samples are incubated with an equal volume of EboBun-GFP in DMEM, at 37°C, 5% CO₂ for 1 hr followed by addition of 150 μl per well of a confluent 12 well plate of VeroE6 cells
10 (MOI = 0.0005). After 2 hours at 37°C, 5% CO₂, 1 ml of DMEM, 2% fetal bovine serym (FBS), 100 U/ml penicillin, 100 μg/ml streptomycin is added per well and incubated for 5 days. Cells are harvested by removing the culture supernatant, washing with 1 ml PBS, 0.04% EDTA, then adding 800 μl of PBS 0.04% EDTA for 5 minutes at 37°C before adding 8 ml PBS, 4% paraformaldehyde (PFA) and overnight incubation. The cells are acquired (10,000 events) and analyzed with CellQuest
15 Pro v3.3 on a Becton Dickinson FACSCalibur flow cytometer.

[0241] The OR and IN routes produce EboBunGP-specific neutralizing antibodies with the OR route producing the highest titres post-vaccination. The IM immunization produces detectable levels of neutralizing antibody. In comparison, 3/4 NHPs in the OR group demonstrate a 50% reduction in EboBun-GFP positive cells at a titre of 1:40. Similarly, the IN route results in a reduction of
20 EboBun-GFP positive cells at the 1:40 dilution.

[0242] EboBunGP-specific effector cellular immune responses are determined using IL-2 and IFN-γ ELISPOT assays as described by Qin, X, et al., PLoS ONE. 2009; 4(5): e5547 to determine the number of IL-2 and IFN-γ secreting lymphocytes. Prior to challenge on days 10 to 14 post-vaccination there is a detectable EboBun immunogen-specific IFN-γ response in all immunized
25 animals. The IM route is the most potent, inducing approximately 2-fold more IFN-γ secreting cells than OR (p<0.001) or IN (p = 0.043) routes. A strong post-challenge secondary IFN-γ response is induced in all VSVΔG/EboBun immunized animals with the IM route producing the most IFN-γ cells at day 6. By day 10 the OR group demonstrates a stronger response. The IFN-γ in the IN group rises steadily, peaking at day 26 post-challenge with 4.3 and 2 fold more EboBun specific
30 IFN-γ secreting cells than the IM (p = 0.003) and OR (p = 0.075) group, respectively. All three routes produce strong EboBun-specific IFN-γ responses.

[0243] Post-vaccination, the IM group also has more EboBunGP-specific IL-2 secreting cells than either of the mucosally immunized groups. Post-challenge, the IM route continues to dominate early after challenge peaking on day 10. This difference shows a trend when compared to the IN group ($p = 0.067$) and is significant when compared to the OR group ($p < 0.001$). Additionally, the IN group has more IL-2 producing cells than the OR group ($p = 0.090$) on day 10 post-challenge. By day 26 post-challenge all three routes continue to produce a EboBunGP-specific IL-2 response, however, the IN group response is strongest. At day 26 post-challenge the IN group has the most potent IFN- γ and IL-2 responses, as well as the highest IgA and IgG antibody titre, indicating this immunization route, followed by a EboBun challenge, results in the development of potent and sustained effector responses.

[0244] Absolute lymphocyte numbers for CD3⁺, CD4⁺, and CD8⁺ (CD3⁺4⁻) T cell populations are determined by flow cytometry. No decrease is observed in the lymphocyte populations for any of the VSV Δ G/EboBunGP vaccinated NHPs. In contrast, control animals who are not protected from EboBun show lymphocyte numbers decreased by 28–57%.

[0245] Macrophage numbers are slightly increased in control animals. However, the number of CD14⁺ cells is greater in the VSV Δ G/EboBunGP vaccinated groups with the IM route showing the most significant increases.

[0246] In order to determine the long term immune response after challenge, EboBunGP-specific CD4⁺ and CD8⁺ memory T-lymphocytes are examined for their ability to proliferate (CFSE⁻) or produce IFN- γ in response to EboBunGP peptides at 6 months post-vaccination. EboBunGP-specific memory responses are observed as a result of vaccination followed by a ZEBOV challenge. These responses persist for at least 6 months. The memory populations in OR and IN inoculation routes demonstrate the greatest potential for proliferation and IFN- γ production post-challenge.

[0247] Any patents or publications mentioned in this specification are incorporated herein by reference to the same extent as if each individual publication is specifically and individually indicated to be incorporated by reference.

[0248] The compositions and methods described herein are presently representative of preferred embodiments, exemplary, and not intended as limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art. Such changes and other uses can be made without departing from the scope of the invention as set forth in the claims. All numerical

ranges are inclusive of the whole integers and decimals between the endpoints, and inclusive of the endpoints.

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CLAIMS

1. An isolated hEbola virus comprising a nucleic acid molecule comprising a nucleotide
5 sequence of:
- a) a nucleotide sequence set forth in SEQ ID NOS: 1 or 10;
 - b) a nucleotide sequence hybridizing under stringent conditions to SEQ ID NOS: 1 or
10; or
 - c) a nucleotide sequence of at least 70%-99% identity to the SEQ ID NOS: 1 or 10.
- 10 2. An isolated hEbola virus having Centers for Disease Control Deposit Accession No.
200706291.
3. The hEbola virus of any one of claims 1 or 2 which is killed.
4. The hEbola virus of claim 1 which is an attenuated hEbola virus.
5. The virus of claim 4 wherein at least one property of the attenuated hEbola virus is
15 reduced from among infectivity, replication ability, protein synthesis ability, assembling ability or
cytopathic effect.
6. An isolated nucleic acid molecule comprising the nucleotide sequence of SEQ ID
NOS: 1 or 10 or a complement thereof.
- 20 7. An isolated nucleic acid molecule comprising a nucleotide sequence of between 4 and
4900 contiguous nucleotides of the nucleotide sequence of SEQ ID NOS: 1 or 10, or a complement
thereof; with the proviso that said nucleotide sequence is not comprised by the nucleotide sequence
set forth in SEQ ID NO: 20; or between 5500 and 6600 contiguous nucleotides of the nucleotide
sequence of SEQ ID NOS: 1 or 10, or a complement thereof.
- 25 8. An isolated nucleic acid molecule comprising a nucleotide sequence that encodes the
amino acid sequence of SEQ ID NO: 2-9, 59, or SEQ ID NO: 11-19 or a complement of said
nucleotide sequence.

9. An isolated RNA or DNA nucleic acid molecule which hybridizes under stringent conditions to a nucleic acid molecule having the nucleotide sequence of SEQ ID NOS: 1 or 10 or a complement thereof.

5 10. An isolated polypeptide encoded by the nucleic acid molecule of any one of claims 7-9.

11. An isolated polypeptide comprising the amino acid of:

a) an amino acid sequence set forth in any of SEQ ID NOS: 2-19, or 59; or

b) an amino acid sequence that has 70% - 99% homology to the amino acid sequence of

(a).

10

12. An isolated polypeptide comprising the amino acid sequence having

5 to 250 contiguous amino acid residues of the amino acid sequence of SEQ ID NOS: 5 or 18 (VP24);

5 to 280 contiguous residues of the amino acid sequence of SEQ ID NOS: 6 or 17 (VP30);

15 5 to 320 contiguous residues of the amino acid sequence of SEQ ID NOS: 8 or 13 (VP40);

5 to 340 contiguous residues of the amino acid sequence of SEQ ID NOS: 7 or 12 (VP35);

5 to 370 contiguous residues of the amino acid sequence of SEQ ID NOS: 4 or 15 (SGP);

5 to 370 contiguous residues of the amino acid sequence of SEQ ID NOS: 59 or 16 (SSGP);

5 to 670 contiguous residues of the amino acid sequence of SEQ ID NOS: 9 or 14 (GP);

20 5 to 730 contiguous residues of the amino acid sequence of SEQ ID NOS: 3 or 11 (NP); or

5 to 2200 contiguous residues of the amino acid sequence of SEQ ID NOS: 2 or 19 (L).

13. An isolated antibody or an antigen-binding fragment thereof which immunospecifically binds to the hEbola virus of any one of claims 1 or 2 or neutralizes the virus.

25

14. An isolated antibody or an antigen-binding fragment thereof which immunospecifically binds to the polypeptide of any one of claims 11 or 12 or neutralizes an hEbola virus.

30 15. A method for detecting the presence of a the hEbola virus or a nucleic acid molecule derived therefrom of claim 1 in a biological sample, said method comprising:

(a) contacting the sample with an agent that selectively binds to the virus or the nucleic acid molecule derived therefrom; and

(b) detecting whether the compound binds to the virus or the nucleic acid molecule derived therefrom in the sample.

5 16. The method of claim 15, wherein the agent is an antibody.

17. The method of claim 15, wherein the agent is a nucleic acid molecule comprising a nucleotide sequence having between 4 and 6600 contiguous nucleotides of the nucleotide sequence of SEQ ID NOS: 1 or 10, or a complement thereof.

10 18. A method for detecting the presence of the polypeptide of claim 11 in a biological sample, said method comprising:

(a) contacting the biological sample with an agent that selectively binds to said polypeptide; and

(b) detecting whether the compound binds to said polypeptide in the sample.

15 19. The method of claim 18, wherein the agent is an antibody or an antigen-binding fragment thereof.

20. A formulation comprising the hEbola virus of any one of claims 3 or 4, and a pharmaceutically acceptable carrier.

20 21. A formulation comprising an amount of a protein extract of the hEbola virus of claim 3 or 4 or a subunit thereof, and a pharmaceutically acceptable carrier.

22. A formulation comprising an amount of a nucleic acid molecule of the nucleotide sequence of SEQ ID NOS: 1 or 10 or a complement thereof, and a pharmaceutically acceptable carrier.

25 23. A formulation comprising an immunogenically effective amount of the nucleic acid molecule of claim 9 or a complement thereof, and a pharmaceutically acceptable carrier.

24. A vaccine formulation comprising a therapeutically or prophylactically effective amount of the hEbola virus of claim 3 or 4 or a protein extract therefrom, and a pharmaceutically acceptable carrier.

25. A vaccine formulation comprising a therapeutically or prophylactically effective amount of a nucleic acid molecule SEQ ID NOS: 1 or 10 or a complement thereof, and a pharmaceutically acceptable carrier.

26. A vaccine formulation comprising a therapeutically or prophylactically effective amount of a nucleic acid molecule of claim 9 or a complement thereof, and a pharmaceutically acceptable carrier.

27. A pharmaceutical composition comprising a prophylactically or therapeutically effective amount of an anti-hEbola agent of an antibody or an antigen-binding fragment thereof which immunospecifically binds to the hEbola virus of Deposit Accession No. 200706291, or polypeptides or protein derived therefrom and optionally has the nucleotide sequence of SEQ ID NOS: 1 or 10, or a fragment thereof.

28. A kit comprising a container containing the formulation of any one of claims 24-26.

29. A method for identifying a subject infected with the virus of claim 1 or 2, comprising:
(a) obtaining total RNA from a biological sample obtained from the subject;
(b) reverse transcribing the total RNA to obtain cDNA; and
(c) amplifying the cDNA using a set of primers derived from a nucleotide sequence of the virus of claim 1 or 2.

30. A primer that has the nucleotide sequence of one of SEQ ID NOS: 24-57.

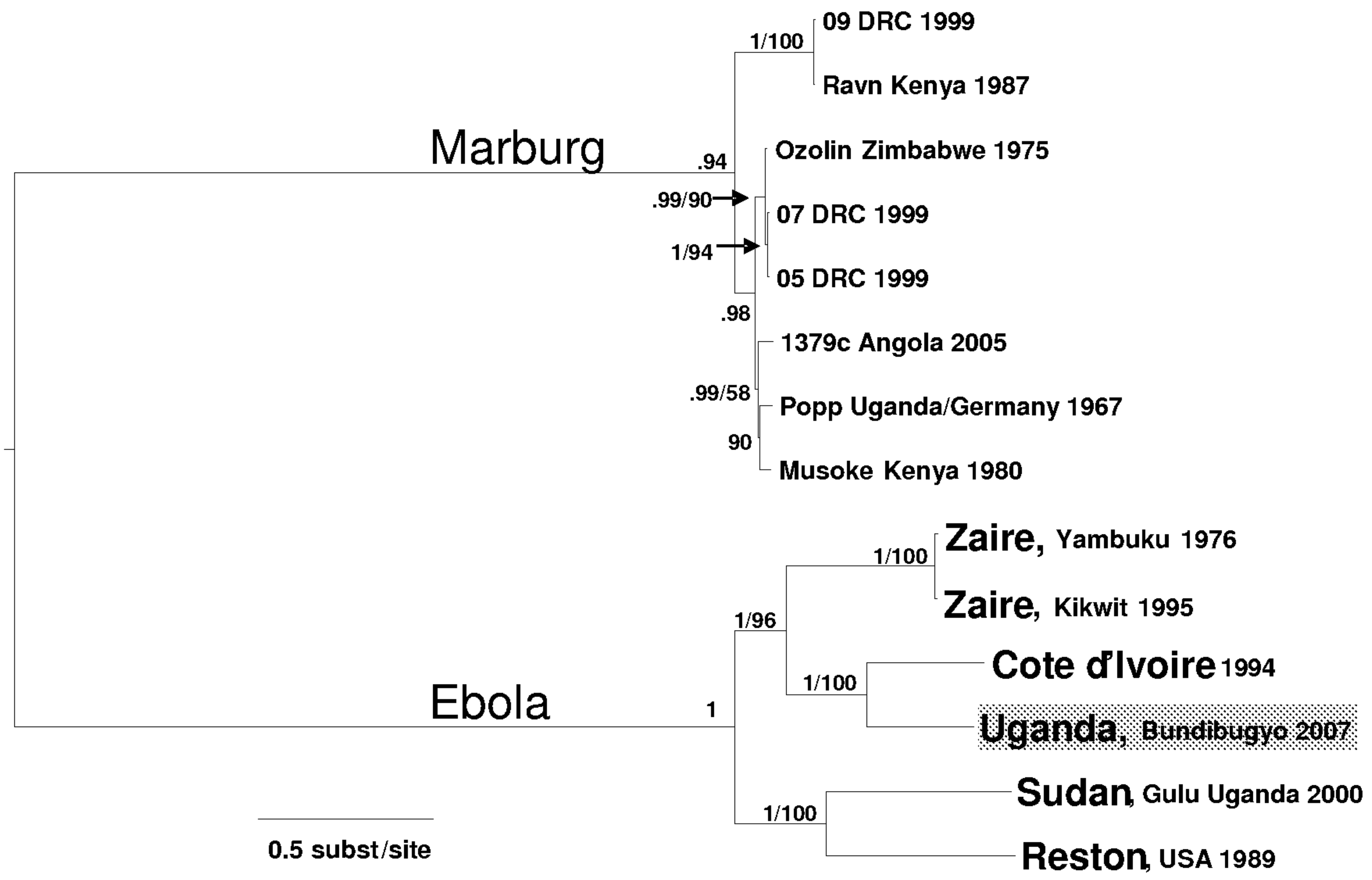


Fig. 1

FIG. 2

	810	820	830	840	850	860	870	880	890	900
Ebola Bundibugyo '07	ATGAAGAAAAGGAGGTCAAGCGCTGGAGGAATACTCCCTGCTGCCTGAGTGAAGAAACAATCAAGAGAACATTTGGCTGCAATGCCCGAGGAGG	Ebola IC '94	GTCAGGAAAAGGAGTCAAGCGACTCAAGAAATTTCTTCCCTGCTGCATCCAGTGGCAAGACATCAGGAGAACACTGGCTGCAATGCCCTGAAGAGG	Ebola Zaire '76	GTCAGAAGCGTGTATGGAGTGAAGCGCTTGAAGAAATTTGAGGAATTTGCTGCCAGCAGTATCTAGTGGAAAACAATTAAGAGAACACTTGTCTGCCATGCCGGAAGAGG		
Ebola Bundibugyo '07	AAACAACAGAAAGCAAAATGCTGGACAATTTCTTTTCATTTGCTAGTCTGTTTCTCCCAAAATGTTGTCGGAGAAAAGGCCCTGTCTGGAGAAAGGTTCAACG	Ebola IC '94	AGACAACAGAAAGCAAAATGCCGGACAGTTCCCTCTCTTTTGTAGTCTTATTTCTTCCCTAAGCTAGTTGTGGAGAAAAGCCCTGTCTAGAAAAGGTTGCAGCG	Ebola Zaire '76	AGACAACAGAAAGCAAAATGCCGGTCAAGTTTCTCTCTTCCGAAATTTGGTAGTAGGAGAAAAGGCCCTTGCCTTGAGAAAAGGTTCAAAAG		
Ebola Bundibugyo '07	ACAAATCCAAAGTGCACGAGAAACAAGGCTGTGATTCATACCCGACATCTTGGCAATCGGTGGGACATATGATGGTCACTTTCAGACTAATGCCGAACCAAC	Ebola IC '94	GCAAAATCAAGTTCAATTTGAGCAGGGATGATCCAAATACCCACAGCCTGGCAGTCAAGTGGACACATGATGGTCAATTTTCAGACTGATGAGAACAAAT	Ebola Zaire '76	GCAAAATCAAGTACATGCAGAGCAAGGACTGATACAAATATCCAAACAGCTTGGCAATCAGTAGGACACATGATGGTGTATTTCCGTTTGTGATGCCGAACAAAT		
Ebola Bundibugyo '07	TTTCTGATTAAGTTCCCTCCTAATACATCAAGGAAATGCATATGTTGTCAGGGCATGATGCTAATGATGCCGTCATTTGCCAACTCTGTAGCTCAAGCTCGTT	Ebola IC '94	TTTCTAAATTAAGTTCCCTCCTTATACATCAAGGATGCATATGTTGTCAGGACACGATGCTAACCATCGCTGTCATCGCAAACTCTGTAGCTCAAGCACGTT	Ebola Zaire '76	TTTCTGATCAAAATTTCTCCTAATACACCAAGGATGCATATGTTGTCAGGGCATGATGCCAACGATGCTGTGATTTCAAATTCAGTGGCTCAAGCTCGTT		
Ebola Bundibugyo '07	TCTCCGGATTTGATAGTCAAAAACAGTGTGATCATATCTCCAAAACAGAGCACGGAGTTCCCTGCATCCCTTTGGCGGAAACAGCCAAAAGTCAA	Ebola IC '94	TTTCAAGGATTAATGATCGTTAAAACAGTGTAGATCAATCCTTCAGAAAACAGAGCACGGAGTGCCTTTCATCCCTTTGGCAAGAACTGCTAAGGTCAA	Ebola Zaire '76	TTTCAAGGCTTATGATTTGTCAAAACAGTGTGATCATATCTCCATAAAAAGACAGAACGGAGTTCCGTCCTCCATCCCTTTGCAAGGACCCGCCAAGGTAAA		
Ebola Bundibugyo '07	AAATGAGGTGAGCTCTTTTAAGGCCGCTTAGCCCTCACATAGCACAACATGGAGAATATGCCCCGTTTGTCTCGTGAATCTATCTGGGGTTAATAAT	Ebola IC '94	GAAACGAGTAAATTCCTTTAAGGCTGCCCTTAGCTCGCTAGCACAACATGGAGAGTATGCTCCTTTTGTCTCGTGAATCTTTCTGGAGTCAACAAT	Ebola Zaire '76	AAATGAGGTGAACCTCTTTAAGGCTGCACCTCAGCTCCCTGGCCAAAGCATGGAGAGTATGCTCCTTTCCGCCGACTTTTGAACCTTTCTGGAGTAAATAAT		
Ebola Bundibugyo '07	CTTGAGCATGGGCTTTTCCCTCAACTTTCTGCAATTTCTGGAGTAGCAACTGCACATGGAGCACTCTGGCTGGAGTCAATGTAGGAGAGCAATACC	Ebola IC '94	CTCGAGCACGGACTGTTTCTCAGCTTTCTGCAATTTGCCCTAGGTTGCGCAACGGCACACGGCAGTACCTTGGCAGGAGTAAATGTGGGGAACAGTATC	Ebola Zaire '76	CTTGAGCATGGTCTTTTCCCTCAACTATCGGCAATTTGCACCTGGAGTCCCTCGCAGGAGTAAATGTGGAGAACAGTATC		

FIG. 2

	1510	1520	1530	1540	1550	1560	1570	1580	1590	1600
									
Ebola Bundibugyo '07	AACA	ACTG	CGA	GAAG	CGC	CACT	GAG	CCG	AAA	AGC
Ebola IC '94	AGCA	ACTA	CGA	GAAG	CGC	CACT	GAG	CCG	AAA	AGC
Ebola Zaire '76	AACA	ACTA	CGA	GAAG	CGC	CACT	GAG	CCG	AAA	AGC
	1610	1620	1630	1640	1650	1660	1670	1680	1690	1700
									
Ebola Bundibugyo '07	AATC	CTAA	AAAG	ACTT	CCAT	CAGA	AAAG	AAAT	GGAT	CCAG
Ebola IC '94	GATC	TTGA	AAAG	ACTT	CCAT	CAGA	AAAG	AAAT	GGAT	CCAG
Ebola Zaire '76	AATC	CTAA	AAAG	ACTT	CCAT	CAGA	AAAG	AAAT	GGAT	CCAG
	1710	1720	1730	1740	1750	1760	1770	1780	1790	1800
									
Ebola Bundibugyo '07	GCTA	TTACT	TCC	ACCT	TAT	CC	TC	CA	AA	AG
Ebola IC '94	GCA	ATC	ACCT	TCC	ACCT	TAT	CC	TC	CA	AA
Ebola Zaire '76	GCTA	TTACT	TCC	ACCT	TAT	CC	TC	CA	AA	AG
	1810	1820	1830	1840	1850	1860	1870	1880	1890	1900
									
Ebola Bundibugyo '07	AGA	ACG	ATG	ACG	ATC	CA	AA	CG	AT	CA
Ebola IC '94	AGC	AA	CG	AT	CA	AA	CG	AT	CA	AA
Ebola Zaire '76	ATC	AA	CG	AT	CA	AA	CG	AT	CA	AA
	1910	1920	1930	1940	1950	1960	1970	1980	1990	2000
									
Ebola Bundibugyo '07	TG	AT	G	CT	G	CA	A	G	T	G
Ebola IC '94	TG	AC	G	CA	A	G	T	G	CA	A
Ebola Zaire '76	AA	AC	G	CA	A	G	T	G	CA	A
	2010	2020	2030	2040	2050	2060	2070	2080	2090	2100
									
Ebola Bundibugyo '07	TAG	AC	CA	AG	TC	TA	CG	AC	CA	AG
Ebola IC '94	CA	AA	CA	AG	TC	TA	CG	AC	CA	AG
Ebola Zaire '76	CAG	AA	CA	AG	TC	TA	CG	AC	CA	AG
	2110	2120	2130	2140	2150	2160	2170	2180	2190	2200
									
Ebola Bundibugyo '07	AG	CC	AA	TG	CC	AA	AA	TC	AT	GC
Ebola IC '94	AT	CC	AA	TG	CC	AA	AA	TC	AT	GC
Ebola Zaire '76	CG	CC	AA	TG	CC	AA	AA	TC	AT	GC

FIG. 2

2210 2220 2230 2240 2250 2260 2270 2280 2290 2300
|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Ebola Bundibugyo '07 CGACCACAACGATGGTGACAAATGAAAGCATTCCTCCCTGGAATCAGACGACGAGG-GTAGCACTGATACTACTGACGAGAAAACAAGCCTGCCACTGC
Ebola IC '94 TGGTCACAAATGAAAGATGACATTTGATAGCATCCCTCCCTTGGAAATCAGACGAAAGAAA-ACAACACTGAGACAACCATTACCACCCACAAAATAACCACTGC
Ebola Zaire '76 GGACGATGCCGACGACGAGACGGTCTAGCCCTTCCGCCCTTGGAGTCAGATGATGAAGACGAGGACGGAACTTCCAAACCCGCACA-CCCACCTGTCTGC

 2310 2320 2330 2340 2350 2360 2370 2380 2390 2400
|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Ebola Bundibugyo '07 ACCTCCCGCTCCCGTCTACCCGAAGTATCTCCGTAGATGATCTGTCCCTCAGAGAACAATTCCTCCGACAGTCCAAATCAAACGAACAATGAGGACAATGTC
Ebola IC '94 TCCACAGCACCTGTGTTTATCGGAGTAAATTCAGAAAAGGAGCCCTCCCGCAAGAAAATCCAGAAAACCAACCAAGTGAAGTGGTAGTGAGAAATACC
Ebola Zaire '76 CCCACCGGCTCCCGTATACAGAGATCACTCTGAAAAGAAAAGAACTCCCGCAAGACGAGCAACAAGATCAGGACCACTCAAGAGGCCAGGAACCAGGAC

 2410 2420 2430 2440 2450 2460 2470 2480 2490 2500
|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Ebola Bundibugyo '07 AGGAACAATGCTCAGTCGGAGCAATCCATTCAGAAATGATCAACATATCTTGAAAACAACAAGGACCTTTTGATGCCATCCTTTACTACCATATGATGA
Ebola IC '94 GACAATAAACCTCACTCAGAGCAATCAGTGGAAAGAAATGATCGACACATCCCTCCAAAACAACAAGGACCATTTTGATGCCATCCTATACATATGATGA
Ebola Zaire '76 AGTGACAACACCCAGTCAGAACACTCTTTTGAGGAGATGATCGCCACATTTCTAAGATCACAGGGGCCATTTTGATGCTGTTTGTATTATCATATGATGA

 2510 2520 2530 2540 2550 2560 2570 2580 2590 2600
|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Ebola Bundibugyo '07 AAGAAGAGCCCATCAATTTTCAGCACATAGTGGAAAGGAGTATACATATCCAGACTCTCTTTGAAGATGAGTATCCACCCTGGCTCAGCGAGAAGGAAGC
Ebola IC '94 CGGAGGAGCCGATGTCTTTAGCACATAGTGGAAAGAAATACCGTATACCTGATCTCTTTGAAGGGGAGCATCCACCCTGGCTCAGTGAAGAAAGAGGC
Ebola Zaire '76 AGGATGAGCCCTGTAGTTTTTCAGTACCAGTGGCAAGAGTACACGTATCCAGACTCCCTTTGAAGAGGAATATCCACCATGGCTCAGTGAAGAAAGAGGC

 2610 2620 2630 2640 2650 2660 2670 2680 2690 2700
|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Ebola Bundibugyo '07 CATGAACGAAGACAAATAGATTCAATAACCATGGATGGTCAGCAGTTTTACTGGCCTGTGATGAATCATAGAAAATAAATTCATGGCAATCCTCCAGCATCAC
Ebola IC '94 CTTGAAATGAGGACAAATAGGTTTATCAACAATGGATGATCAACAATTTACTGGCCTGTAATGAATCACAGGAACAATAATTCATGGCTATCCTTCAGCACCCAC
Ebola Zaire '76 TATGAATGAAGAGAAATAGATTTTGTACATTTGATGGATGGTCAACAATTTTATTTGGCCGGTGTGATGAATCACAAAGATAAATTCATGGCAATCCTGCAACATCAT

 2710 2720 2730 2740 2750 2760 2770 2780 2790 2800
|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Ebola Bundibugyo '07 AGGTGATCCGACCTCTAAAACCTGAGCT---CCTAACCTACAAGCTAC-CCCATCACTCTGCCCAGGAAATGCCAGAACCTCCCTCCAAAACAGCTCCACATCGA
Ebola IC '94 AAGTAAATTTCTTCAATAATGACAGATCA---TTGTAAAGTTATTACC-ACCATCCCTGCAACAAGCATGAAAACCACTCAACAACGCCCTTACCACAGG
Ebola Zaire '76 CAGTGAATGAGCATGGAACAATGGGATGATTCAAACCGACAAATAGCTAACAATTAAGTAGTCAAGGAACGAAAAC-----AGGAAGAATTTTGTGATGTC

 2810 2820 2830 2840 2850 2860 2870 2880 2890 2900
|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Ebola Bundibugyo '07 GAACCTCCGACCGGTACACAGGCAAGACAGGCAACCTAAATGATGTTCTGTTCCACCCACAACCGCAACCAACACTTGATCGACTTCCAAGAC--AACTA
Ebola IC '94 ATACCTTGGAGACCAATACACCAAGATCAGCAGCTGTGCAACCACCCCTCCATGGAAATCCACCAACCAACCAACCAAT--AATCCCAAGACCAAAACCG
Ebola Zaire '76 TAAGGTGTAATTATTATCACAAATAAAGTGATT-CITATTTTTGAATTTAAAGCTAGCTTATTATTACTAGCCGTTTTTCAAAAGTTCAATTTGAGTCTT

FIG. 2

Ebola Bundibugyo '07
 Ebola IC '94
 Ebola Zaire '76
 2910 2920 2930 2940 2950 2960 2970 2980 2990 3000
|.....|.....|.....|.....|.....|.....|.....|.....|.....|
 CAACCCCTTAGC--CAACTCCACACAGAGCACACCCCAAAACCAA-CAACACTGCATGTAAGTATTGTCTCACCCCAAGATGA
 CACACATCCAGAT--CAACCCAAACCCCTCAAAACACCCACCCCTCCG-CGATCCAGACCAAACTCCGCCCCAGACAAGCACCCACCCATCCAGAAAC
 AATGCAAATAGGGGTTAAGCCACAGTTATAGCCATAAATGTAACTCAAT-ATTCTAACTAGCGATTATCTAAATTAATAATACATTAATGCTTTTATAACT

Ebola Bundibugyo '07
 Ebola IC '94
 Ebola Zaire '76
 3010 3020 3030 3040 3050 3060 3070 3080 3090 3100
|.....|.....|.....|.....|.....|.....|.....|.....|.....|
 TCCCTGGACACCAACACCCCTAAACCTCCCAAGTTGTCAATTAAGAAAAAATAATATGATGAAGATTAATAAACCTTCAATCAGAGCTATTTCTTCTACGCTT
 CGCACGGCCGAGAAATCGATCCAGCATTCAAAATGCGTTATTAAGAAAAACAATATGATGAAGATTAATAAACCTTCAATCAACATTCACAGACTTTGATC
 TACCTACTAGCCTGCCCAACATTTACACGATCGTTTATATAATTAAGAAAAAACTAATGATGAAGATTAATAAACCTTCAATCATCCTTACGTTCAATTGAATTC

Ebola Bundibugyo '07
 Ebola IC '94
 Ebola Zaire '76
 3110 3120 3130 3140 3150 3160 3170 3180 3190 3200
|.....|.....|.....|.....|.....|.....|.....|.....|.....|
 GGTTAGGACCAAGTATTCACAAAATAATTTACAATC-----CTTACCCTAATATGACCTTAACAGAGCAAGGGTGAATTAACAACCCACCCCAACCAACCA
 CTTAGGAGTTTATCTAGCTATCTACAAAACGGGT-----CCAAAACGGAATGATTTCCACTAGGGCTGCAGCAATCAATGATCCTTTCATTAACCAATCA
 TCTAGCACTCGAAGCTTATGTCTTCAATGTAAAGAAAGCTGGTCTAACCAAGATGACAACTAGAACAAAGGGCAGGGCCATATACTGGGCCACCGACTC

Ebola Bundibugyo '07
 Ebola IC '94
 Ebola Zaire '76
 3210 3220 3230 3240 3250 3260 3270 3280 3290 3300
|.....|.....|.....|.....|.....|.....|.....|.....|.....|
 CAGGCACACGATCGTGTGGCCGGAACTTCCGGGTGGATCTGTAGCAATTGATGACAGGCAAGATTCGGATTACCGATATCTTCAATGAAATTGAAAC
 GAAACCAAGTGTACACAGTGGCCCTGAACATACAGGATGGATCTCCGAAACAATTAATGACAGGCAAAATTCGGGTACATGAAATCTTCAACGACACTGAGCC
 AAAACGACAGAAATGCCAGGCCCTGAGCTTCCGGCTGGATCTCTGAGCAGCTAATGACCGGAAGAAATTCCTGTAAGCGGACATCTTCTGTGATATTGAGAA

Ebola Bundibugyo '07
 Ebola IC '94
 Ebola Zaire '76
 3310 3320 3330 3340 3350 3360 3370 3380 3390 3400
|.....|.....|.....|.....|.....|.....|.....|.....|.....|
 CTTACCTAGTATAAGTCCCTCGATCCACTCCAAAATCAAAACCCCAAGTGTTCAAAACACGCGAGTGTCCAGACCCCAAACTGACCCCAAAATTTGTAATCATGAT
 CCACATAAGCTCAGGGTCCGACTGCCTTCCAGACCCAAAACACGGCCCTCCGACTCGCAACACCCAGACACAGACCCGATCCGGTTTGCATCACAAT
 CAATCCAGGATTTATGCTACGCATCCCAAATGCAACAACGAAAGCCAAACCCGAAAGACCGCAACAGTCAAAACCCCAACCCCAAAATTTGCAATCATAGT

Ebola Bundibugyo '07
 Ebola IC '94
 Ebola Zaire '76
 3410 3420 3430 3440 3450 3460 3470 3480 3490 3500
|.....|.....|.....|.....|.....|.....|.....|.....|.....|
 TTTGCAGAGGTTGTGAAAATGCTAACAATCTTAACCCCTTGTCTGTAACAAAACCAAAACCCCTTGCAACTGAATCACTTGAAGCAACCGCATTAAGCTGGAAG
 TTTGAAGACGTTACACAAGCACTAACAATCAATTAACCAATGTCAATCAAAAACAGGCTCTTAACCTTAGAGTCTCTCGAACAACCGCATCATAGATCTAGAGA
 TTTGAGGAGGTAGTACAAAACATTTGGCTTCAATTTGGCTACTGTTGTGCAACAACAACCACTCGCATCAGAAATCATTAGAACAAACCGCATTAAGCTTTGAGA

Ebola Bundibugyo '07
 Ebola IC '94
 Ebola Zaire '76
 3510 3520 3530 3540 3550 3560 3570 3580 3590 3600
|.....|.....|.....|.....|.....|.....|.....|.....|.....|
 GTAGCCTGAAACCAAGTGTCTGAGATCACCAAGATTTGTTCTGCACTAAATAGATCCTGTGCAAGATGGTGGCCAAAATATGATCTTCTAGTAATGACGAC
 ATGGCTTAAAGCCAAATGATGACATGGCTAAAGTCAATTTCTGCAATGAATAGATCTTTGTGCTGAGATGGTAGCAAAAATATGATCTCCTGGTATGACAAAC
 ATGGTCTAAAGCCAGTTTATGATATGATATGGCAAAAACAATCTCCTCATTTGAACAGGGTTTGTGCTGAGATGGTTGCAAAAATATGATCTTCTGGTATGACAAAC

FIG. 2

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5910      |.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Ebola Bundibugyo '07      GTAAA-TTGTATGGTATCAAACTTATTAAGAAAAGAAACACGATGAAGATTAACGCGACCTAGAGCGCTGCCCTTCATCTCAATTAACCTTGTCAA
Ebola IC '94              ACGCACTTCTTATGCCA-CAGCTTATATAAGAAAAGAAACTTGATGAAGATTAAGGCAACCAGTGGTCTATCTTCATCTCTTTGATTTGAGTCTTAAG
Ebola Zaire '76           ATCCAAGTACAGACATTGCCCTTCTAATTAAGAAAATAATCGCGGATGAAGATTAAGCCGACAGTGAGCGGTAATCTTCATCTCTCTTAGATTAATTTGTTTT
6010      |.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Ebola Bundibugyo '07      TAGAGCAACCTAGTTTGTGATTACTCATCT-TCCGTAGTTGACAAAACACTTTGCTGGTTAATTGTAATAATACCACAGTCAATGGTTACATCAGGAAT
Ebola IC '94              TGAATACACAGGTTCTAATACTGTCTTCTGTCCACAGGTAATAATCAGCCAGGCTAAGACAGTAGCTA-ATCACAGTCAATCATGGGAGCGTCAAGGAT
Ebola Zaire '76           CCAGAGTAGGGTCTGTCAGTCCCTTTTCAATCGTGTAAACAAAATAAACTCCACTAGAAGGATATTGTGGGCAACAA-CACAATGGGCGTTACAGGAAT
6110      |.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Ebola Bundibugyo '07      TCTACAAATGCCCCGTTGAACGTTTCAGAAAACATCATTTTTTTGGTAAATTAATCCTATTTCACAAAAGTTTTCCCTATCCCATGGGCGTAGTTCCAC
Ebola IC '94              TCTGCAATGGCCCGTGAGCGCTTCAGGAAAACATCTTTTGTGGTAAATAATCCTATTCCATAAAGTCTTTTCAATCCCGTTGGGGTTGTACAC
Ebola Zaire '76           ATTGCAGTTACCTCGTGATCGATTCAAGAGGACATCATCTTTCTTTGGGTAATAATCCTTTTCCAAAGAACATTTTCCATCCCACTTGGAGTCAATCCAC
6210      |.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Ebola Bundibugyo '07      AACAACTCTCCAGGTAAGTGATATAGATAAATGGTGTGCCGGGATAAACTTTCCACAAAGTCAGCTGAAATCGGTCGGCTTAATCTAGAAGGTA
Ebola IC '94              AACAACTCCCTACAAGTGAGTGATATTGACAAGTTTGTGTGCCGAGACAAAACCTCTCTTCAACTAGCCAAATGAAGTCAGTCGGTTGAACTTGGAGGGCA
Ebola Zaire '76           AATAGCACATTACAGGTTAGTGATGTGACAAAACCTAGTTTGTGTCGACAAAACCTGTCATCCACAAAATCAATGAAGTGGTCAATGAAATCTCCGAAGGGA
6310      |.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Ebola Bundibugyo '07      ATGGAGTTGCCACAGATGTACCACCAAGCAAGAGATGGGATTCAGAGCTGGTGTCCACCCCAAAGTGGTGAACCTACGAAGCTGGGAGTGGGCTGA
Ebola IC '94              ATGGAGTAGCAACTGATGTACCACCAAGCAAGAGATGGGATTCAGAGCTGGTGTCCACCCCAAAGTGGTGAATTCGAAGCTGGGAGTGGGCTGA
Ebola Zaire '76           ATGGAGTGGCAACTGACGTGCCATCTGCAACTAAAGATGGGCTTCAGGTCCGGTGTCCACCAAAGTGGTCAATTAAGAGCTGGTGAATGGGCTGA
6410      |.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Ebola Bundibugyo '07      AAACCTGCTACAACCTGGACATCAAGAAAAGCAGATGGTAGCGAATGCCCTACCTGAAGCCCTGAGGGTGAAGAGGCTTCCCTCGCTGCTTATGTGCAC
Ebola IC '94              GAACTGTTATAAACCCTGGCTATAAAGAAAAGTTGATGGTAGTGAGTGCCTACCAGAAAGCCCTGAGGGAGTGAGGGATTTTCCCGCTGCGCTATGTACAC
Ebola Zaire '76           AAACCTGCTACAATCTTGAATCAAAAACCTGACGGGAGTGAGTGTCTACCAGCAGCCGCGGATTCGGGGCTTCCCGGTTCCCGGTTATGTGCAC
6510      |.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Ebola Bundibugyo '07      AAGGTTTCTGGAAACAGGGCCGTGCCCTGAAGGTTACGCTTTCACAAAAGAGCCGCTTTCCTGTATGATCGACTGGCATCAACAATCATCTATCGAA
Ebola IC '94              AAAGTCTCAGGAACTGGACCATGCCCAGGAGGACTCGCCTTTCACAAAAGAGGAGCCTTTCCTGTATGACCGACTCGCATCAACAATCATTTATCGGG
Ebola Zaire '76           AAAGTATCAGGAAACGGGACCGTGTGCCGGAGACTTTGCTTTCCTGTATGATCGACTTGTCTCCACAGTTGTCCACAGTTATCTACCCGAG

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FIG. 2

7410 7420 7430 7440 7450 7460 7470 7480 7490 7500
|.....|.....|.....|.....|.....|.....|.....|.....|.....|
 Ebola Bundibugyo '07 CCGCCAGTCCCAACAGCACAATCCATCCACACACAGAGACTCACATTCACCAACAAAGCCATGACACCCGACAGCAATCGACCCCAAC
 Ebola IC '94 CCCACGGTCCCAACACACAGAAAGCCAGCCGAACTTGGCAAGAACCCCAACACACTCCAGAACAGCACACTGCCGCCAGTCCATTCCAAGA
 Ebola Zaire '76 GAGCAAGACTGACTTCTCTGGACCCCGCCACCAACAAGTCCCAAAACACAGCGAGACCGCTGGCAACAACAACACTCATCCAAAGATACCCGGA

7510 7520 7530 7540 7550 7560 7570 7580 7590 7600
|.....|.....|.....|.....|.....|.....|.....|.....|.....|
 Ebola Bundibugyo '07 CCAATTGACATCAGCGAGTCTACAGAGCCAGGACCACTCACCAACACACAGAGGGCTGCAAAATCTGCTGACAGGCTCAAGAAGAACCCGAAGGAAA
 Ebola IC '94 GCCGTGACCCCGACGAACCTCAGTGGACCTGGCTTCCTGACGAAACAATAACGGGGGTTACAAATCTCCTGACAGGATCCAGAAGAAAGCGAAGGATG
 Ebola Zaire '76 GAAGAGAGTGCCAGCAG--CGGGAAGCTAGGCTTAATTACCAATACTATTGCTGGAGTCCAGGACTGATCACAGCGGGGAGAAACTCGAAGAGAAG

7610 7620 7630 7640 7650 7660 7670 7680 7690 7700
|.....|.....|.....|.....|.....|.....|.....|.....|.....|
 Ebola Bundibugyo '07 TCACCCCTGAGAACACAAAGCCAAATGCAACCCAAACCTACACTATTGGCAACCCCAAGATGAAGGGCTGCCATTTAGCTGGATACCTTACTTCCGG
 Ebola IC '94 TCACCTCCCAATACACAAACCCAAATGCAACCCAAACCTGCACTATTGGACAGCCTTGGATGAGGGTGTGCCATAGGTTTAGCTGGATACCACTACTTCCGG
 Ebola Zaire '76 CAATTGTCAATGCTCAACCCAAATGCAACCCCTAAATTTACATTACTGGACTACTCAGGATGAAGGTGCTGCAATCGGACTGGCTGGATACCAATATTCCGG

7710 7720 7730 7740 7750 7760 7770 7780 7790 7800
|.....|.....|.....|.....|.....|.....|.....|.....|.....|
 Ebola Bundibugyo '07 GCCCGCAGCAGAGGGAAATTTATACGGAAAGGATAATGCACAAATCAAAATGGGCTAAATTTGCGGGTTGAGGCAGCTAGCAAAATGAGACGACTCAAGCCCTA
 Ebola IC '94 GCCAGCAGCTGAGGGAAATTTACACTGAAGGCATAAATGGAGAAATCAAAATGGATTTGATCTGTGGATGAGGCAGCTGGCCAAACGAAACGACACAAGCTCTT
 Ebola Zaire '76 GCCAGCAGCCGAGGGAAATTTACATAGAGGGGCTAAATGCACAAATCAAGATGTTTAAATCTGTGGTTGAGACAGCTGGCCAAACGAGACGACTCAAGCTCTT

7810 7820 7830 7840 7850 7860 7870 7880 7890 7900
|.....|.....|.....|.....|.....|.....|.....|.....|.....|
 Ebola Bundibugyo '07 CAGTTATTCTTGGCTGTACCACGGAAATGGCCACTTCTCTATATTGAATCGAAAAGCCATCGACTTTTACTCCAAAGATGGGGAGAAACGTTGCCACA
 Ebola IC '94 CAATTGTTCTTAAGGGCAACTACTGAGTTGCGGTACTCTATATACTAAATCGGAAAGCAATAGACTTCTTGTCTCCAAAGATGGGGAGAAACATGTACACA
 Ebola Zaire '76 CAACTGTTCTGTGAGGCCACAACCTGAGCTACGCACCTTTTCAATCTCAACCGTAAAGCAATTTGATTTCTTGTGTCAGCGATGGGGCGGCACATGCCACA

7910 7920 7930 7940 7950 7960 7970 7980 7990 8000
|.....|.....|.....|.....|.....|.....|.....|.....|.....|
 Ebola Bundibugyo '07 TCTTAGGCCCAGATTGCTGTATTGAGCCCCATGATGGACTAAGAACAATTACTGACAAAAATAGATCAAAATCATTCATGATTCATGATAAACCTCTACC
 Ebola IC '94 TTC'TAGGGCCTGATTGTTGCAATTGAACCCCAAGATTGAGTGGACCAAAAATATCACTGATAAAAATGATCAAAATATCCATGACTTTTGTGATAAATAATCTTCC
 Ebola Zaire '76 TTC'TGGACCCGGACTGCTGTATCGAACCAATGATGGACCAAAAATGATCAAGATTTTGTGATAAACCCTTCC

8010 8020 8030 8040 8050 8060 8070 8080 8090 8100
|.....|.....|.....|.....|.....|.....|.....|.....|.....|
 Ebola Bundibugyo '07 AGATCAAAACAGATAAATGACAAATTTGGTGGACAGGGTGGAGGCAATGGGTTCCCTGCCGGGATCGGGATCACGGGGTAAATAATCCAGTTATAGCAGCTGCTG
 Ebola IC '94 AAATCAGAAATGATGGCAGCAACTGGTGGACTGGAACAATGGGTTCCCTGCTGGAATAGGAATCAGAGGATAATCATTTGCTATTTATGCTTTGCTG
 Ebola Zaire '76 GGACCAGGGGACAAATGACAAATTTGGTGGACAGGATGGAGACAATGGATACCGGAGGATTTAGGTTTACAGGCTTATAAATGCAGTTATCGCTTTATTC

FIG. 2

10410 10420 10430 10440 10450 10460 10470 10480 10490 10500
Ebola Bundibugyo '07|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Ebola IC '94 AACAGATCGATACTAATAAGGATTTGTT-TCATACTAGCTCTCTGCAAAACACAATGGCTAAGGCAACAGGTAGGTACAACACTTGGTTTCACCTAAAAA
Ebola Zaire '76 AGTGAACAAGCTCATACTATCAAGGCTAATCCTCGGGCTGCCTGGAGTC--CACAATGGCCAAAGGCTACTGGGAGGTACAACCTTATCTCCCCAAAGAA
AGACAACAAAATATTGATACT---CCAGACACCAAGCAAGACCTTGAGAAAAAACCAATGGCTAAAGCTACGGGACGATACAATCTAATATCGCCCAAAAA

10510 10520 10530 10540 10550 10560 10570 10580 10590 10600
Ebola Bundibugyo '07|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Ebola IC '94 GGACCTCGAGAGGGGCTTTTGTAGTGATTTGTGCACCGTTTTTAGTTGATCAGACTATCCAGGGGTGGCGGGTGACTTGGGTTGGGATTTGAATTTGAC
Ebola Zaire '76 AGATCTTGA AAAAGGGCTGGTTCTGAAATGACCTTTGCACTCTCTCAGTGGCCAGACGGTCCAGGGATGGAAGTTACCTGGGCTGGGATTTGAATTTGAT
GGACCTGGAGAAAAGGGTTGTCTTAAGCGACCTCTGTAACCTTTAGTTAGCCAAACTATTCAGGGGTGGAAGGTTTATTGGGCTGGTATTGAGTTTGTAT

10610 10620 10630 10640 10650 10660 10670 10680 10690 10700
Ebola Bundibugyo '07|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Ebola IC '94 ATCGCCAGAAAAGGATGGCTCTACTGTCATCGGTTAAAAACTGCTGACTTCGCTCCTGTCATGGTCGATGACAAGGAATTTATTTCCCTCATTTATTTCAAA
Ebola Zaire '76 GTTACACAGAAAAGGATGGCCCTTATTGCACAGGCTCAAGACCAGTGAATTTGCTCCAGCTGGTCAATGACCAGGAACCTTATTTCCACATCTCTTTTCAAA
GTGACTCACAAAAGGAATGGCCCTATTGTCATAGACTGAAAACCTAATGACTTTGCCCTGTCATGGTCAATGACAAGGAATCTCTTTCCCTCATTTATTTCAAA

10710 10720 10730 10740 10750 10760 10770 10780 10790 10800
Ebola Bundibugyo '07|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Ebola IC '94 ATTCAAATTTCTACTATTGAGTCTCCCTCTGGGCAATTACGAGTGAATCTGGCAGCTGGTATTCAAGACCAGTTAATTGACCAATCCTTTGGTAGAACCCGTT
Ebola Zaire '76 ACCCGAATCTACAATGAGTGGCCACTTTGGGCACTGCGGTCATACTAGCAGCAGGATTTCAAGATCAGCTAATGATCAATCGTTGATCGAACCCCTT
ATCCGAAATCCACAATTTGAATCACCCGCTGTGGCATTGAGAGTCACTCCCTTGCAGCAGGGATACAGGACCAGCTGATGACCAGTCTTTTGTATTGAAACCCCTT

10810 10820 10830 10840 10850 10860 10870 10880 10890 10900
Ebola Bundibugyo '07|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Ebola IC '94 GGCCGGAGCCCTGAGCTTAGTCTCCGATTTGGCTTTTCAACAACAACAACCACTTTTCAATGGCCAGCAGCACGCTAAAGAGCAACTGAGCTTGAAG
Ebola Zaire '76 GGCAGGAGCCCTAGGCTTAATTGCTGATTTGGCTTTTACTACTGGAACAACAACCACTTTCAATGGCCACACAACAGGCTAAAGGACCACTAAGTCTAAAA
AGCAGGAGCCCTTGGTCTGATCTCTGATTTGGTCTGCTAACAACAACCACTAACCATTTCACAATGGGAACACACACAGTGTCAAGGAACAATTTGAGCCATAAAA

10910 10920 10930 10940 10950 10960 10970 10980 10990 11000
Ebola Bundibugyo '07|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Ebola IC '94 ATGCTATCATTAGTGGCTCTAATAATCTTGAATTCATCAGTCAATTTGGACGCCTACTACATGTCGTGAACCTACAATGGACTCTTTGAGCAGTATCGAAAATTG
Ebola Zaire '76 ATGTTGTCCCTGGTGGATCAACCATCTAAAGTTTCATCAACCAACTAGATGCACCTACATGTTGTGAATTTACAATGGACTTCTCAGTAGCATTTGAAAATTG
ATGCTGTCTGATTCTGATCCCAATATTCTCAAGTTTATTAACAATAATTTGATGCTCTACATGTCGTGAACCTACAACCGGATTTGTTGAGCAGTATTGAAAATTG

11010 11020 11030 11040 11050 11060 11070 11080 11090 11100
Ebola Bundibugyo '07|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Ebola IC '94 GCAC TAGAAAATCATACCATTTATCATCAAGAACAACCATGGGTTTCTCGTAGAATTTACAGGAGCCCTGATAAATCTGCCATGAACTCAAAAAGAAAACCAGG
Ebola Zaire '76 GCACCAAAAGCCATACAATTAATAATCCCGGACAATAATGGGTTTTTTGGTAGAGTTGCAAGAGCCCTGACAAAATCAGCCATGAACACCAGAAAACCAGG
GAACTCAAAAATCATACAATCATATAACTCGAACTAATCATGGGTTTTCTGTGGAGCTCCAAGAACCAGCAAAATCGGCAATGAACCGCATGAAGCCCTGG

FIG. 2

	11910	11920	11930	11940	11950	11960	11970	11980	11990	12000
Ebola Bundibugyo '07	Ebola IC '94	AATAGTTACATGCGGATAGATTACTTACCCCTTACGAACTTATCCGGGAGGCTTATGCCCTGTGAAACCAGGTCAGCCAAATCTTA	Ebola Zaire '76	GGTAGCAACATGCGGAGGATTTTGTAGTACATTTCTTAGGACTCTATCAGGAAATGGTTCTTGTCCAAATGATCCAAAATGCAGTCAATTTTAA		AGTGGCGACATGCGCCATAGATTTCATAGTCCAGTTCCTCAAGGCACGTGTGAGGCAATGGATTCTGTCTGTGAGCCCGGGTGCCAAACAGTTCTTA			
	12010	12020	12030	12040	12050	12060	12070	12080	12090	12100
Ebola Bundibugyo '07	Ebola IC '94	GATGAAAATAGTAAGTTATGTTTGCAGGATGCACGTTTAAAGATACTATTTTAGGCATGTTGGAGTACACGATGACAATGTTGGAAAAAATTTTGAGC	Ebola Zaire '76	GAAGAAAATGTCAAATTAACCTACAAAGATATCGCTTCCATAAATACCTCAATCGAGCCGGAGTGCATAACGATCATGTGGATAGGATTTTGAC		GATGAAAATCAATTAAGTACACAATGCAAGATGCTCTCTTTGAAAATATTAATCTCAAAAATGTGGTGTCTCAAGAAGACTGTGTTGATGAACACTTTTCAAG			
	12110	12120	12130	12140	12150	12160	12170	12180	12190	12200
Ebola Bundibugyo '07	Ebola IC '94	CAAAAGATTAAGGCTTTGATTAATGATAATGAAATTTCTGCAACAATTTGTTTATTTGGTACGATTTAGCAATCCCTAAACCGCTAGAGGGCCCTGAAATCGAGG	Ebola Zaire '76	AAAAAATTCGCAATCTAATTTGCGCAATGAGGTTTACATCAATGTTTCACTGGTATGATCTTGCATTTCTAGCACGCTAGAGGGCGACTAAATAGAGG		AGAAAATCTTATCTTCAATTCAGGGCAATGAAATTTTACATCAATGTTTCTTGGTATGATCTGGCTATTTTAACTCGAAAGGGTAGATTAATTCGAGG			
	12210	12220	12230	12240	12250	12260	12270	12280	12290	12300
Ebola Bundibugyo '07	Ebola IC '94	GAAATAACCGTTCAACATGGTTTGCAAAATGACGATTTAATAGACATTTCTCGGGTACGGTGATTAATTTCTGGAAAATACCGTTGTCAATGTTGTCACTC	Ebola Zaire '76	GAATAATCGCTCAACATGGTTTGCAAAATGAGTATCTAGGATTAATTTTGGAGATTAATTTTGGAGAAATACCAATTAATCACTACCTACCCAGTG		AAACTCTAGATCAACATGGTTTGTTCATGATGATTAATAGACATCTTAGGCTATGGGCACTATGTTTTTGGAAAGATCCCAATTTCAATGTTTACCACCTG			
	12310	12320	12330	12340	12350	12360	12370	12380	12390	12400
Ebola Bundibugyo '07	Ebola IC '94	AACACAGAGGGGATTCCTCATGCGACTAAGGACTGGTATCACGCATCAATCTTCAAGAAGCGGTTCAAGGTCACACACATATCGTGTCAAGTTTCCACTG	Ebola Zaire '76	GATACACAAGGCCCTCCACATGCGCAAGGACTGGTATCATGAATCGGTTTCAAGGAGGCTATTCAGGCCATACACACATCGTGTCCATCTCTACAG		AACACACAAGGAAATCCCCATGCTATGACTGGTATGACTGAGGCAATCAAGGATTAATCAAGGATTAATCAAGGCTATGTTTCTGTGTTTCTACTG			
	12410	12420	12430	12440	12450	12460	12470	12480	12490	12500
Ebola Bundibugyo '07	Ebola IC '94	CAGATGTTTAAATTAATGTAAGGACATCAATAACCTGTGTTTCAATACCCACACTCATGCGCAATGCGCAATTTAGAAGATTTCTATCTGTCTGACTA	Ebola Zaire '76	CAGATGCTTAAATCATGTAAGGACATAAATCACCTGTGATTTAATACCTTTACTGATTTGCTGTGGCAATCTAGAGGATTCAGTTTCATTCAGATTA		CCGACGCTTGATAAATGTGCAAAAGATTTAATTAATACATGCTGATTTCAACAACACTTAATCTCAAAAATAGCAGAGATTGAGGATCCAGTTTGTCTGATTA			
	12510	12520	12530	12540	12550	12560	12570	12580	12590	12600
Ebola Bundibugyo '07	Ebola IC '94	TCCACAACCTGAAAACAATCTCTAATCTGTAAGGCAGGGGATTAATCTCGATACTGGGTTTCAGAAGGTTATAAGGTCATAAAGTTTGTAGAACCA	Ebola Zaire '76	CCCTTACCAGAAAACAGTGTCTGACCTATACAAAAGCAGGAGATTAATTTAAATCTCATTTGCTAGGATCAGAAGGTTACAAGTCAAAAATTCCTTGAGCCG		TCCCAATTTAAGATTTGTCTATGCTTTTACCAGAGCGGAGATTAATTAATTAAGGTTAGGTTCTGATGGGTTAATAAATTAATTAAGTTCTCTCGAACCA			

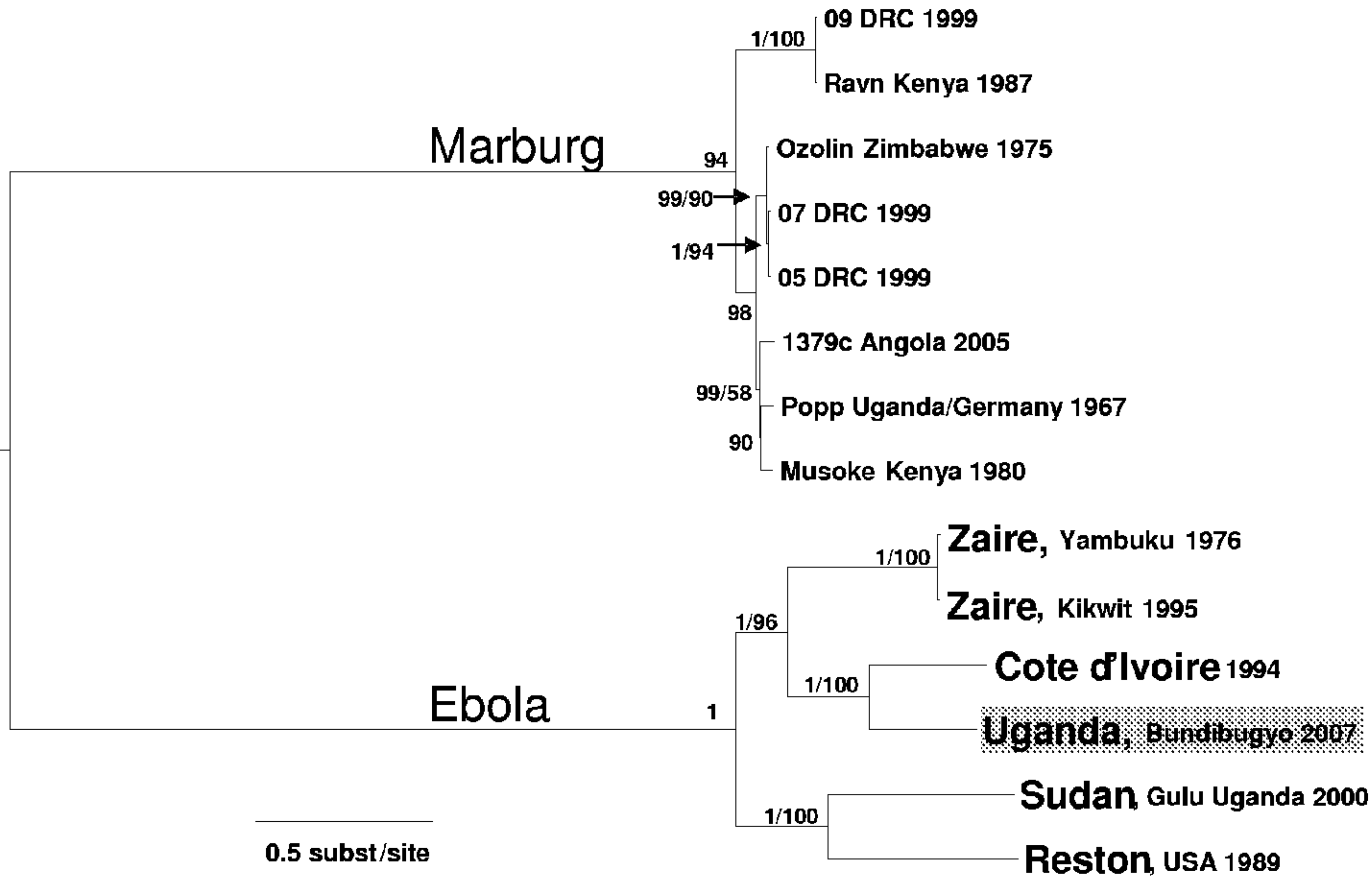


Fig. 1