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Kawaoka et al. et al.

(54) **RECOMBINANT BIOLOGICALLY CONTAINED FILOVIRUS VACCINE**

(71) Applicant: **Wisconsin Alumni Research Foundation (WARF)**, Madison, WI (US)

(72) Inventors: **Yoshihiro Kawaoka et al.**, Middleton, WI (US); **Peter J. Halfmann**, Madison, WI (US)

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(57) **ABSTRACT**

The invention provides a vaccine comprising a recombinant biologically contained filovirus and methods of making and using those viruses.

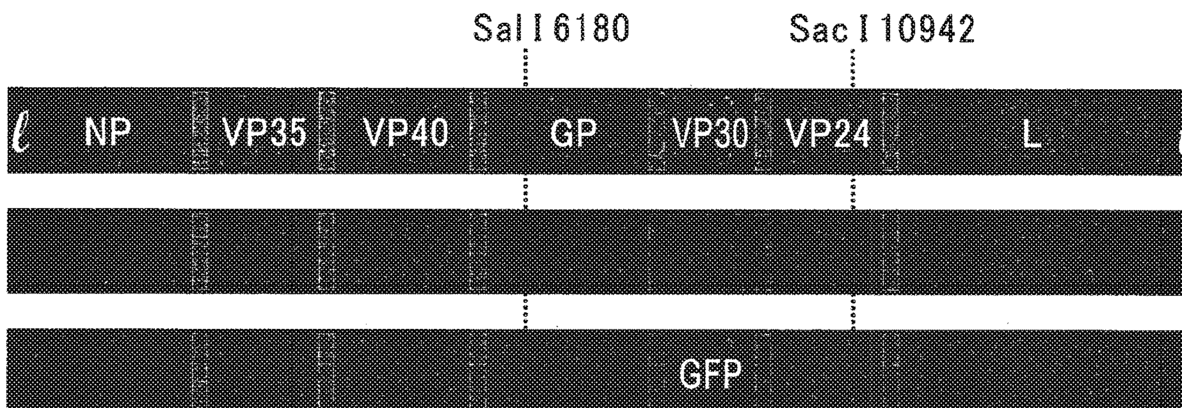

FIG. 1

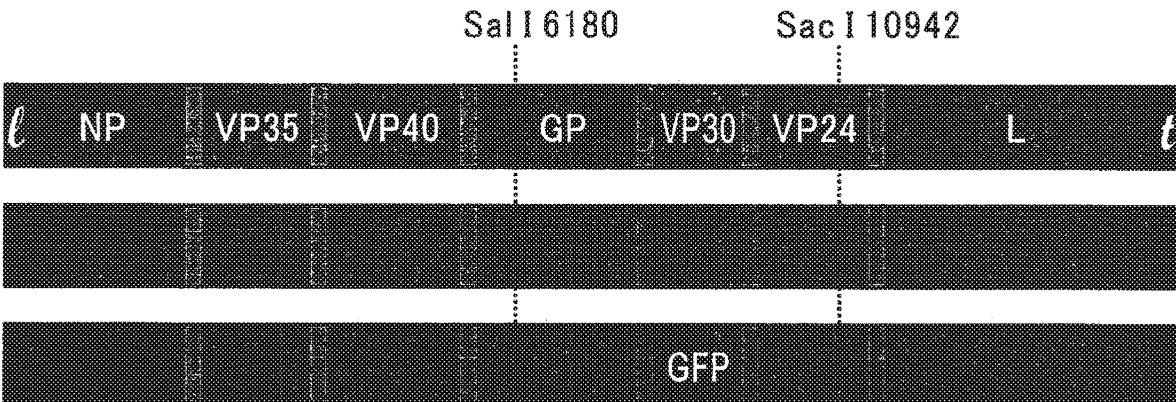

Figure 2

NC_006432

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AY142960

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AF272001

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11161 catctctgct ttcataatca gatatataag cataataaat aaatactcat atttcttgat
11221 aatttgttta accacagata aatcctcact gtaagccagc ttccaagttg acacccttac
11281 aaaaaccagg actcagaatc cctcaaacaa gagattccaa gacaacatca tagaattgct
11341 ttattatatg aataagcatt ttatcaccag aaatcctata tactaaatgg ttaattgtaa
11401 ctgaacccgc aggtcacatg tgttaggttt cacagattct atatattact aactctatac
11461 tcgtaattaa cattagataa gtagattaag aaaaaagcct gaggaagatt aagaaaaact
11521 gcttattggg tctttccgtg ttttagatga agcagttgaa attcttcctc ttgatattaa
11581 atggctacac aacataccca atacccagac gctaggttat catcaccaat tgtattggac
11641 caatgtgacc tagtcactag agcttgcggg ttatattcat catactccct taatccgcaa
11701 ctacgcaact gtaaactccc gaaacatatc taccgtttga aatacgatgt aactgttacc
11761 aagttcttga gtgatgtacc agtggcgaca ttgcccatag atttcatagt cccagttctt
11821 ctcaaggcac tgtcaggcaa tggattctgt cctgttgagc cgcggtgcca acagttctta
11881 gatgaaatca ttaagtacac aatgcaagat gctctcttct tgaaatatta tctcaaaaat
11941 gtgggtgctc aagaagactg tgttgatgaa cactttcaag agaaaatctt atcttcaatt
12001 cagggcaatg aatttttaca tcaaatgttt ttctggtatg atctggctat tttaactcga
12061 aggggtagat taaatcgagg aaactctaga tcaacatggt ttgttcatga tgatttaata
12121 gacatcttag gctatgggga ctatgttttt tggaagatcc caatttcaat gttaccactg
12181 aacacacaag gaatccccca tgctgctatg gactggtatc aggcatcagt attcaaagaa
12241 gcggttcaag ggcatacaca cattgtttct gtttctactg ccgacgtctt gataatgtgc
12301 aaagatttaa ttacatgtcg attcaacaca actctaatct caaaaatagc agagattgag
12361 gatccagttt gttctgatta tcccaatttt aagattgtgt ctatgcttta ccagagcgga
12421 gattacttac tctccatatt agggtctgat gggtataaaa ttattaagtt cctcgaacca
12481 ttgtgcttgg ccaaaattca attatgctca aagtacactg agaggaaggg ccgattctta
12541 acacaaatgc atttagctgt aaatcacacc ctagaagaaa ttacagaaat gcgtgcacta
12601 aagccttcac aggctcaaaa gatccgtgaa ttccatagaa cattgataag gctggagatg

12661 acgccacaac aactttgtga gctattttcc attcaaaaac actgggggca tcctgtgcta
12721 catagtgaaa cagcaatcca aaaagttaaa aaacatgcta cggtgctaaa agcattacgc
12781 cctatagtga ttttcgagac atactgtgtt tttaaatata gtattgccaa acattatttt
12841 gatagtcaag gatcttggta cagtgttact tcagatagga atctaacacc gggtcttaat
12901 tcttatatca aaagaaatca attccctccg ttgccaatga ttaaagaact actatgggaa
12961 ttttaccacc ttgaccaccc tccactttc tcaaccaaaa ttattagtga cttaagtatt
13021 tttataaaag acagagctac cgcagtagaa aggacatgct gggatgcagt attcgagcct
13081 aatgttctag gatataatcc acctcacaaa tttagtacta aacgtgtacc ggaacaattt
13141 ttagagcaag aaaacttttc tattgagaat gttctttcct acgcacaaaa actcgagtat
13201 ctactaccac aatatcggaa cttttctttc tcattgaaag agaaagagtt gaatgtaggt
13261 agaaccttcg gaaaattgcc ttatccgact cgcaatgttc aaacactttg tgaagctctg
13321 ttagctgatg gtcttgctaa agcatttcct agcaatatga tggtagttac ggaacgtgag
13381 caaaaagaaa gcttattgca tcaagcatca tggcaccaca caagtgatga ttttggtgaa
13441 catgccacag ttagagggag tagctttgta actgatttag agaaatacaa tcttgcattt
13501 agatatgagt ttacagcacc ttttatagaa tattgcaacc gttgctatgg tgttaagaat
13561 gtttttaatt ggatgcatta tacaatccca cagtgttata tgcatgtcag tgattattat
13621 aatccaccac ataacctcac actggagaat cgagacaacc cccccgaagg gcctagttca
13681 tacaggggtc atatgggagg gattgaagga ctgcaacaaa aactctggac aagtatttca
13741 tgtgctcaaa tttctttagt tgaaattaag actggtttta agttacgctc agctgtgatg
13801 ggtgacaatc agtgcattac tgttttatca gtcttcccct tagagactga cgcagacgag
13861 caggaacaga gcgccgaaga caatgcagcg agggtggccg ccagcctagc aaaagttaca
13921 agtgcctgtg gaatcttttt aaaacctgat gaaacatttg tacattcagg ttttatctat
13981 tttggaaaaa aacaatattt gaatggggtc caattgcctc agtcccttaa aacggctgca
14041 agaatggcac cattgtctga tgcaattttt gatgatcttc aagggaccct ggctagtata
14101 ggcactgctt ttgagcgatc catctctgag acacgacata tctttccttg caggataacc
14161 gcagctttcc atacgttttt ttcggtgaga atcttgcaat atcatcatct cgggttcaat
14221 aaaggttttg accttggaca gttaacactc ggcaaacctc tggatttcgg aacaatatca
14281 ttggcactag cggtaccgca ggtgcttgga gggttatcct tcttgaatcc tgagaaatgt
14341 ttctaccgga atctaggaga tccagttacc tcaggcttat tccagttaaa aacttatctc
14401 cgaatgattg agatggatga tttattctta cctttaattg cgaagaaccc tgggaactgc
14461 actgccattg actttgtgct aaatcctagc ggattaaatg tccctgggtc gcaagactta
14521 acttcatttc tgcgccagat tgtacgcagg accatcaccc taagtgcgaa aaacaaactt
14581 attaatacct tatttcatgc gtcagctgac ttcgaagacg aaatggtttg taaatggcta
14641 ttatcatcaa ctcctgttat gagtcgtttt gcggccgata tcttttcacg cacgccgagc
14701 gggaagcgat tgcaaattct aggatacctg gaaggaacac gcacattatt agcctctaag
14761 atcatcaaca ataatacaga gacaccggtt ttggacagac tgaggaaaat aacattgcaa
14821 aggtggagcc tatggtttag ttatcttgat cattgtgata atatcctggc ggaggcttta
14881 acccaaataa cttgcacagt tgatttagca cagattctga gggaatattc atgggctcat
14941 attttagagg gaagacctct tattggagcc acactcccat gtatgattga gcaattcaaa
15001 gtgttttggc tgaaacccta cgaacaatgt ccgcagtgtt caaatgcaaa gcaaccaggt
15061 gggaaaccat tcgtgtcagt ggcagtcaag aaacatattg ttagtgcatg gccgaacgca
15121 tcccgaataa gctggactat cggggatgga atcccataca ttggatcaag gacagaagat
15181 aagataggac aacctgctat taaaccaaaa tgtccttccg cagccttaag agaggccatt
15241 gaattggcgt cccgtttaac atgggtaact caaggcagtt cgaacagtga cttgctaata
15301 aaaccatttt tggaagcacg agtaaattta agtgttcaag aaatacttca aatgacccct
15361 tcacattact caggaaatat tgttcacagg tacaacgatc aatacagtcc tcattctttc

15421 atggccaatc gtatgagtaa ttcagcaacg cgattgattg tttctacaaa cactttaggt
15481 gagttttcag gaggtggcca gtctgcacgc gacagcaata ttattttcca gaatgttata
15541 aattatgcag ttgcactgtt cgatattaaa tttagaaaca ctgaggctac agatatccaa
15601 tataatcgtg ctcaccttca tctaactaag tgttgcaccc gggaagtacc agctcagtat
15661 ttaacataca catctacatt ggatttagat ttaacaagat accgagaaaa cgaattgatt
15721 tatgacagta atcctctaaa aggaggactc aattgcaata tctcattcga taatccattt
15781 ttccaaggta aacggctgaa cattatagaa gatgatctta ttcgactgcc tcacttatct
15841 ggatgggagc tagccaagac catcatgcaa tcaattattt cagatagcaa caattcatct
15901 acagacccaa ttagcagtgg agaaacaaga tcattcacta cccatttctt aacttatccc
15961 aagataggac ttctgtacag ttttggggcc tttgtaagtt attatcttgg caatacaatt
16021 cttcggacta agaaattaac acttgacaat tttttatatt acttaactac tcaaattcat
16081 aatctaccac atcgctcatt gcgaatactt aagccaacat tcaaacatgc aagcgttatg
16141 tcacggttaa tgagtattga tcctcatttt tctatttaca taggcggtgc tgcaggtgac
16201 agaggactct cagatgcggc caggttattt ttgagaacgt ccatttcatc ttttcttaca
16261 tttgtaaaag aatggataat taatcgcgga acaattgtcc ctttatggat agtatatccg
16321 ctagagggtc aaaacccaac acctgtgaat aattttctct atcagatcgt agaactgctg
16381 gtgcatgatt catcaagaca acaggctttt aaaactacca taagtgatca tgtacatcct
16441 cacgacaatc ttgtttacac atgtaagagt acagccagca atttcttcca tgcatcattg
16501 gcgtactgga ggagcagaca cagaaacagc aaccgaaaat acttggcaag agactcttca
16561 actggatcaa gcacaaacaa cagtgatggt catattgaga gaagtcaaga acaaaccacc
16621 agagatccac atgatggcac tgaacggaat ctagtcctac aaatgagcca tgaaataaaa
16681 agaacgacaa ttccacaaga aaacacgcac cagggtccgt cgttccagtc ctttctaagt
16741 gactctgctt gtggtacagc aaatccaaaa ctaaatttcg atcgatcgag acacaatgtg
16801 aaatttcagg atcataactc ggcatccaag agggaaggtc atcaaataat ctcacaccgt
16861 ctagtcctac ctttctttac attatctcaa gggacacgcc aattaacgtc atccaatgag
16921 tcacaaaccc aagacgagat atcaaagtac ttacggcaat tgagatccgt cattgatacc
16981 acagtttatt gtagatttac cggtatagtc tcgtccatgc attacaaact tgatgaggtc
17041 ctttgggaaa tagagagttt caagtcggct gtgacgctag cagagggaga aggtgctggt
17101 gccttactat tgattcagaa ataccaagtt aagaccttat ttttcaacac gctagctact
17161 gagtccagta tagagtcaga aatagtatca ggaatgacta ctcctaggat gcttctacct
17221 gttatgtcaa aattccataa tgaccaaatt gagattattc ttaacaactc agcaagccaa
17281 ataacagaca taacaaatcc tacttggttt aaagaccaaa gagcaaggct acctaagcaa
17341 gtcgaggtta taaccatgga tgcagagaca acagagaata taaacagatc gaaattgtac
17401 gaagctgtat ataaattgat cttacaccat attgatccta gcgtattgaa agcagtggtc
17461 cttaaagtct ttctaagtga tactgagggt atgttatggc taaatgataa tttagccccg
17521 tttttgccca ctggttattt aattaagcca ataacgtcaa gtgctagatc tagtgagtgg
17581 tatctttgtc tgacgaactt cttatcaact acacgtaaga tgccacacca aaaccatctc
17641 agttgtaaac aggtaatact tacggcattg caactgcaaa ttcaacgaag cccatactgg
17701 ctaagtcatt taactcagta tgctgactgt gagttacatt taagttatat ccgccttggt
17761 tttccatcat tagagaaagt actataccac aggtataacc tcgtcgattc aaaaagaggt
17821 ccactagtct ctatcactca gcacttagca catcttagag cagagattcg agaattaact
17881 aatgattata atcaacagcg acaaagtcgg actcaaacat atcactttat tcgtactgca
17941 aaaggacgaa tcacaaaact agtcaatgat tatttaaaat tctttcttat tgtgcaagca
18001 ttaaaacata atgggacatg gcaagctgag tttaagaaat taccagagtt gattagtgtg
18061 tgcaataggt tctaccatat tagagattgc aattgtgaag aacgtttctt agttcaaacc
18121 ttatatttac atagaatgca ggattctgaa gttaagctta tcgaaaggct gacagggctt

18181 ctgagtttat ttccggatgg tctctacagg tttgattgaa ttaccgtgca tagtatcctg
18241 atacttgcaa aggttggtta ttaacataca gattataaaa aactcataaa ttgctctcat
18301 acatcatatt gatctaatct caataaacaa ctatttaaat aacgaaagga gtccctatat
18361 tatatactat atttagcctc tctccctgcg tgataatcaa aaaattcaca atgcagcatg
18421 tgtgacatat tactgccgca atgaatttaa cgcaacataa taaactctgc actctttata
18481 attaagcttt aacgaaaggt ctgggctcat attgttattg atataataat gttgtatcaa
18541 tatcctgtca gatggaatag tgttttggtt gataacacaa cttcttaaaa caaaattgat
18601 ctttaagatt aagtttttta taattatcat tactttaatt tgtcgtttta aaaacggtga
18661 tagccttaat ctttgtgtaa aataagagat taggtgtaat aaccttaaca tttttgtcta
18721 gtaagctact atttcataca gaatgataaa attaaaagaa aaggcaggac tgtaaaatca
18781 gaaatacctt ctttacaata tagcagacta gataataatc ttcgtgttaa tgataattaa
18841 gacattgacc acgctcatca gaaggctcgc cagaataaac gttgcaaaaa ggattcctgg
18901 aaaaatggtc gcacacaaaa atttaaaaat aaatctattt cttctttttt gtgtgtcca (SEQ ID NO:32)

VP30
MEASYERGRPRAARQHSRDGHDHHVRARSSSRENYRGEYRQSRSASQVRVPTV
FHKKRVEPLTVPPAPKDICPTLKKGFLCDSSFCKKDHQLESLTDRELLLLIARKTC
GSVEQQLNITAPKDSRLANPTADDFQQEEGPKITLLTLIKTAEHWARQDIRTIEDS
KLRALLTLCAVMTRKFSKSQLSLLCETHLRREGLGQDQAEPVLEVYQRLHSDKG
GSFEAALWQQWDRQSLIMFITAFLNIALQLPCESSAVVVSGLRTLVPQSDNEEAS
TNPGTCSWSDEGTP (SEQ ID NO:33)

Bundibugyo Ebola virus polymerase

1 matqhtqypd arlsspivld qcdlvtracg lyssysslnpq lkncrlpkhi yrlkfdatvt
61 kflsdvpivt lpidyltpll lrtlsgeglc pvepkcsqfl deivsyvlqd arflryyfrh
121 vgvhddnvgk nfepkikali ydneflqqlf ywydlailtr rgrlnrgnnr stwfanddli
181 dilgygdyif wkiplsllsl ntegiphaak dwyhasifke avqghthivs vstadvlimc
241 kdiitcrfnt tliaalanle dsicsdypqp etisnlykag dylisilgse gykvikflep
301 lclakiqlcs nyterkgrfl tqmhlavnht leeliegrgl ksqqdwkmre fhrilvnlks
361 tpqqlcelfs vqkhwghpvl hsekaiqkvk khatvikalr pviifetycv fkysiakhyf
421 dsqgswysvi sdkhltpglh syikrnqfpp lpmikdllwe fyhldhpplf stkiisdlsi
481 fikdratave ktcwdavfep nvlgysppnk fstkrvpeqf leqenfsids vltyaqrldy
541 llpqyrnfsf slkekelnvg rafgklpypt rnvqtlceal ladglakafp snmmvvtere
601 qkesllhqas whhtsddfge natvrgssfv tdlekynlaf ryeftapfie ycnrcygvkn
661 lfnwmhytip qcyihvsdyy npphgvslen redppegpss yrghlggieg lqqklwtsis
721 caqislveik tgfklrsavm gdnqcitvls vfpletdsne qehssednaa rvaaslakvt
781 sacgiflkpd etfvhsgfiy fgkkqylngv qlpqslktat riaplsdaif ddlqgtlasi
841 gtafersise trhvypcrvv aafhtffsvr ilqyhhlgfn kgtdlgqlsl skpldfgtit
901 lalavpqvlg glsflnpekc fyrnlgdpvt sglfqlrtyl qminmddlfl pliaknpgnc
961 saidfvlnps glnvpgsqdl tsflrqivrr titlsaknkl intlfhssad ledemvckwl
1021 lsstpvmsrf aadifsrtps gkrlqilgyl egtrtllask vinnnaetpi ldrlrkitlq
1081 rwslwfsyld hcdqvladal ikvsctvdla qilreytwah ilegrqliga tlpcmleqfn
1141 vfwlksyeqc pkcaksrnpk gepfvsiaik kqvvsawpnq srlnwtigdg vpyigsrted
1201 kigqpaikpk cpsaalreai eltsrltwvt qgganlsdllv kpfvearvnl svqeilqmtp
1261 shysgnivhr yndqysphsf manrmsnsat rlvvstntlg efsgggqsar dsniifqnvi

1321 nfsvalfdlr frntetssiq hnrahlhlsq cctrevpaqy ltytstlsld ltryreneli
1381 ydnnplkggl ncnlsfdnpl fkgqrlniie edlirfphls gwelaktiiq siisdsnnss
1441 tdpissgetr sftthfltyp kvgllysfga ivsyylgnti irtkkldlsh fmyylttqih
1501 nlphrslril kptfkhvsvi srlmsidphf siyiggtagd rglsdatrlf lrvaissflq
1561 fikkwiveyk taiplwviyp legqnpdpin sflhliiall qnespqnniq fqedrnnqql
1621 sdnlvymcks tasnffhasl aywrsrhkgr pknrsteeqt vkpipydnfh svkcasnpps
1681 ipksksgtqg ssaffekley dkerelptas tpaeqsktyi kalssriyhg ktpsnaakdd
1741 sttskgcdsk eenavqashr ivlpffltsq ndyrtpsakk seyiteitkl irqlkaipdt
1801 tvycrftgvv ssmhykldev lwefdsfkta vtlaegegsg allllqkykv rtiffntlat
1861 ehsieaeivs gtttprmllp vmaklhddqi nvilnnsasq vtditnpawf tdqksriptq
1921 veimtmdaet teninrskly eaiqqlivsh idtrvlkivi ikvflsdieg llwlndhlap
1981 lfgsgylikp itsspkssew ylclsnflsa srrphqgha tcmqviqtal rlqvqrssyw
2041 lshlvqyadi nlhlsyvnlg fpslekvlyh rynlvdsrkg plvsilyhlt hlqaeirelv
2101 cdynqqrqsr tqtyhfiktt kgritklvnd ylkfylvvqa lkhnclwqee lrtlpdlinv
2161 cnrfyhirdc scedrfliqt lyltrmqdse aklmerltgf lglypngina (SEQ ID NO: 34)

Marburgvirus polymerase

1 mqhptqypda rlsspiildq cdllarslgl yshyshnpkl rncriphhiy rlrnstalkt
61 flqncsiltv pfhsiwdhil tsiqydainh vddfkyllps elvkyanwdn eflkaylnki
121 lgldhvfsas arsqcedfsp kenpyywgml llvhlsqlar rikgqrgslr snwkfigtdl
181 elfgiadfvi fkvpvktiir navslqaskp glriwyrdqn ltpylcddef ivsvasyecf
241 imikdvfier yntweicara wledsdgady ppldvlgely nqgdqiiamy ledgfklikh
301 leplcvsciq thgiftprky wfqsqmiksy ydelhdlnlk lqisdnkaec aqnfiktivq
361 akltpqqyce lfslqkhwgh pvlyndvald kvkkhaqstk ilkpkvmfet fcvfkfivak
421 nhyhsqgswy ktthdlhltp ylrqhivsns fpsqaeiyqh lwewyfvehe plfstkiisd
481 lsifikdrlt avnqecwdsv fdrsvlgynp pvrfqskrvp eqflgqadfs lnqilefaek
541 leylapsyrn fsfslkekel nigrtfgklp yrvrnvqtla ealladglak afpsnmmvvt
601 ereqkeallh qaswhhnsas igenaivrga sfvtdlekyn lafryeftrh fidycnrcyg
661 vknlfdwmhf liplcymhvs dfyspphcvt ednrnnppdc anayhyhlgg ieglqqklwt
721 ciscaqitlv elktklklks svmgdnqcit tlslfpidap ndyqeneaei naarvavela
781 ittgysgifl kpeetfvhsg fiyfgkkqyl ngvqlpqslk tmarcgplsd sifddlqgsl
841 asigtsferg tsetrhifps rwiasfhsml ainllnqnhl gfplgfnidi scfkkpltfs
901 ekliialitpq vlgglsflnp eklfyrnisd pltsglfqlk naleflekee lfyiliskkp
961 gladasdfvm nplglnvprs keiitflrqt vrenititsq nriinslfhi gsdledqrvc
1021 ewllssnpvm srfaadifsr tpsgkrlqvl gylegtrtll asrtisltte gtmlmklrel
1081 trnrwkswfs yidaldddls eslekftctv dvanflrays wsdvlkgkrl igatlpclle
1141 qfevkwinls edlreqfnls sdskstinll pydckelrle gsndtelnyv scaldrkvvq
1201 khpsvnrlaw tignrapyig srtedkigyp plrvncpsaa lkeaiemvsr llwvtqgtad
1261 reklliplln srvnldyqtv lnflpthysg nivhryndqy gqhsfmanrm sntstraiis
1321 tntlgkyagg gqaaidsnii fqntinlgva vldialslak lssasnvtfr lmlnkcctrh
1381 vpseylyfdk pldvdlnkym dnelvydndp lcsgikgrlg rvsrstlsls lnvsdigsyd
1441 fptiaawtlg etivgsifsd essqstdpis sgctktfvth flvypvesif yafganlive
1501 slslsriksi knlsdltfli sstirnlshr slrilqstfr helvltrlah hiplislmlg
1561 gsageksssd avrlfltasy qnfinnfscl mkkgqsslpv wlyfpsegqq lkpilkilqr
1621 lsdllspdki qkrkiladtc cpigsfwvyp skstrtnhyy aslnywrdka nkvkntpfsh

1681 lincsfpefs shtssvssnq qvtnskyivy peniteinar trlinygsta lqgmdtkmpl
1741 seqnlvencr psegirfkdn qkitkhdqrc ereesspqqm fpednmqtpa hihssspfqi
1801 liksldahed fdaskiilns einnlnltey tlntklltp trteildtsp lqssryssts
1861 rersllsreq asylyvdcsn ipsisldpgf rsmsdqnqvq mlintykrdl hacfdsnqfc
1921 rftgvvssmh yklydllppg klkkaiclae gegsgarlll kwketdylff ntlatdsqqe
1981 aeilsgrvip rmlynidrls allesrrlil nnltiqitdi tnplwldsvi qylpedsdil
2041 tmdaettkde treqlyktiv niwtrtspni pkisiikvfl ldyegtlflm knaiqyygqv
2101 qlkkpyssna knsewylccg krriqrlqid fsdqvgifli ckamsrqrqa ipywlkhiek
2161 nypaslheff ltlgfpsles sfchrytipf segkalfhkv qsyvrqgkqh lhslmldyen
2221 nsplldlrnh ficslrgkit kyyndilkln lvikavekgk nwsqlveilp nmhsvcivhv
2281 dhecsgcekr lllkldfirn tkiaeqklln rvigyilffp fglfksgslr a (SEQ ID NO:35)

Zaire Ebola virus polymerase

1 matqhtqypd arlsspivld qcdlvtracg lyssyslnpq lrncklpkhi yrlkydvtvt
61 kflsdvpvat lpidfivpvl lkalsgngfc pveprcqqfl deiikytmqd alflkyylkn
121 vgaqedcvde hfqekilssi qgneflhqmf fwydlailtr rgrlnrgnsr stwfvhddli
181 dilgygdyvf wkipismlpl ntqgiphaam dwyqasvfke avqghthivs vstadvlimc
241 kdlitcrfnt tliskiaeie dpvcsdypnf kivsmlyqsg dyllsilgsd gykiikflep
301 lclakiqlcs kyterkgrfl tqmhlavnht leeitemral kpsqaqkire fhrtlirlem
361 tpqqlcelfs iqkhwghpvl hsetaiqkvk khatvlkalr pivifetycv fkysiakhyf
421 dsqgswysvt sdrnltpgln syikrnqfpp lpmikellwe fyhldhpplf stkiisdlsi
481 fikdratave rtcwdavfep nvlgynpphk fstkrvpeqf leqenfsien vlsyaqkley
541 llpqyrnfsf slkekelnvg rtfgklpypt rnvqtlceal ladglakafp snmmvvtere
601 qkesllhqas whhtsddfge hatvrgssfv tdlekynlaf ryeftapfie ycnrcygvkn
661 vfnwmhytip qcymhvsdyy npphnltlen rdnppegpss yrghmggieg lqqklwtsis
721 caqislveik tgfklrsavm gdnqcitvls vfpletdade qeqsaednaa rvaaslakvt
781 sacgiflkpd etfvhsgfiy fgkkqylngv qlpqslktat rmaplsdaif ddlqgtlasi
841 gtafersise trhifpcrit aafhtffsvr ilqyhhlgfn kgfdlgqltl gkpldfgtis
901 lalavpqvlg glsflnpekc fyrnlgdpvt sglfqlktyl rmiemddlfl pliaknpgnc
961 taidfvlnps glnvpgsqdl tsflrqivrr titlsaknkl intlfhasad fedemvckwl
1021 lsstpvmsrf aadifsrtps gkrlqilgyl egtrtllask iinnntetpv ldrlrkitlq
1081 rwslwfsyld hcdnilaeal tqitctvdla qilreyswah ilegrpliga tlpcmieqfk
1141 vfwlkpyeqc pqcsnakqpg gkpfvsvavk khivsawpna srisvtigdg ipyigsrted
1201 kigqpaikpk cpsaalreai elasrltwvt qgssnsdlli kpflearvnl svqeilqmtp
1261 shysgnivhr yndqysphsf manrmsnsat rlivstntlg efsgggqsar dsniifqnvi
1321 nyavalfdik frnteatdiq ynrahlhltk cctrevpaqy ltytstldld ltryreneli
1381 ydsnplkggl ncnisfdnpf fqgkrlniie ddlirlphls gwelaktimq siisdsnnss
1441 tdpissgetr sftthfltyp kigllysfga fvsyylgnti lrtkkltldn flyylttqih
1501 nlphrslril kptfkhasvm srlmsidphf siyiggaagd rglsdaarlf lrtsissflt
1561 fvkewiinrg tivplwivyp legqnptpvn nflyqivell vhdssrqqaf kttisdhvhp
1621 hdnlvytcks tasnffhasl aywrsrhrns nrkylardss tgsstnnsdg hiersqeqtt
1681 rdphdgtern lvlqmsheik rttipqenth qgpsfqsfls dsacgtanpk lnfdrsrhnv
1741 kfqdhnsask reghqiishr lvlpfftlsq gtrqltssne sqtqdeisky lrqlrsvidt
1801 tvycrftgiv ssmhykldev lweiesfksa vtlaegegag allliqkyqv ktlffntlat
1861 essieseivs gmttprmllp vmskfhndqi eiilnnsasq itditnptwf kdqrarlpkq

1921 vevitmdaet teninrskly eavykliIhh idpsvlkavv lkvflsdteg mlwlndnlap
1981 ffatgylikp itssarssew ylcltnflst trkmphqnhl sckqviltal qlqiqrspyw
2041 lshltqyadc elhlsyirlg fpslekvlyh rynlvdskrg plvsitqhla hlraeirelt
2101 ndynqqrqsr tqtyhfirta kgritklvnd ylkfflivqa lkhngtwqae fkklpelisv
2161 cnrfyhirdc nceerflvqt lylhrmqdse vklierltgl lslfpdglyr fd SEQ ID NO:36

Reston Ebola virus polymerase

1 matqhtqypd arlsspivld qcdlvtracg lyssyslnpq lrqcklpkhi yrlkfdtivs
61 kflsdtpvat lpidylvpil lrsltghgdr pltptcnqfl deiinytlhd aafldyylka
121 tgaqdhltni atreklknei lnndyvhqlf fwhdlsilar rgrlnrgnnr stwfvhdefi
181 dilgygdyif wkiplsllpv tidgvphaat dwyqptlfke silghsqils vstaeilimc
241 kdiitcrfnt sliasiakle dvdvsdypdp sdilkiynag dyvisilgse gykiikylep
301 lclakiqlcs kfterkgrfl tqmhlsvind lrelisnrrl kdyqqekird fhkillqlql
361 spqqfcelfs vqkhwghpil hsekaiqkvk rhatilkalr pnvifetycv fkyniakhyf
421 dsqgtwysvi sdrnltpgln sfikrnhfps lpmikdllwe fyhlnhpplf stkvisdlsi
481 fikdratave qtcwdavfep nvlgynppnk fstkrvpeqf leqedfsies vlnyaqelhy
541 llpqnrnfsf slkekelnig rtfgklpylt rnvqtlceal ladglakafp snmmvvtere
601 qkesllhqas whhtsddfge natvrgssfv tdlekynlaf ryeftapfie ycnhcygvrn
661 vfnwmhylip qcymhvsdyy npphnvnlsn reyppegpss yrghlggieg lqqklwtsis
721 caqislveik tgfklrsavm gdnqcitvls vfplktdpee qeqsaednaa rvaaslakvt
781 sacgiflkpd etfvhsgfiy fgkkqylngv qlpqslktaa rmaplsdaif ddlqgtlasi
841 gtaferaise trhilpcriv aafhtyfavr ilqyhhlgfn kgidlgqlsl skpldygtit
901 ltlavpqvlg glsflnpekc fyrnfgdpvt sglfqlrvyl emvnmkdlfc plisknpgnc
961 saidfvlnps glnvpgsqdl tsflrqivrr sitltarnkl intlfhasad ledemvckwl
1021 lssnpvmsrf aadifsrtps gkrlqilgyl egtrtllask iinnnsetpv ldklrkitlq
1081 rwnlwfsyld hcdqlladal qkisctvdla qilreytwsh ilegrsliga tlpcmveqfk
1141 vkwlgqyepc pecinkkgsn ayvsvavkdq vvsawpntsr iswtigsgvp yigsrtedki
1201 gqpaikprcp ssalkeaiel asrltwvtqg gsnseqlirp flearvnlsv sevlqmtpsh
1261 ysgnivhryn dqysphsfma nrmsntatrl ivstntlgef sgggqaards niifqnvinl
1321 avalydirfr ntntsdirhn rahlhltecc tkevpaqylt ytsalnldls ryrdneliyd
1381 snplkgglnc nltidsplvk gprlnmiedd llrfphlsgw elaktvvqsi isdnsnsstd
1441 pissgetrsf tthfltypqi gllysfgavl cfylgntilw tkkldyeqfl yylhnqlhnl
1501 phralrvfkp tfkhasvmsr lmeidsnfsi yiggtsgdrg lsdaarlflr taiasflqfl
1561 kswiidrqkt iplwivyple gqqpesinef lhkilgllkq gpksipkevs iqndghldla
1621 ennyvvynsks tasnffhasl aywrsrksrk tqdhndfsrg dgtltepvrk fssnhqsdek
1681 yynvtcgksp kpqerkdfsq yrlsnngqtm snhrkkgkfh kwnpckmlme sqrgtvlteg
1741 dyfqnntppt ddvssphrli lpffklgnhn hahdqdaqel mnqnikqylh qlrsmldtti
1801 ycrftgivss mhykldevll eynsfdsait laegegsgal lllqkystrl lflntlateh
1861 siesevvsgf stprmllpim qkvhegqvtv ilnnsasqit ditssmwlsn qkynlpcqve
1921 iimmdaette nlnrsqlyra vynlildhid pqylkvvvlk vflsdiegil windylaplf
1981 gagylikpit ssarssewyl clsnlistnr rsahqthkac lgvirdalqa qvqrgvywls
2041 hiaqyatknl hceyiglgfp slekvlyhry nlvdtglgpl ssvirhltnl qaeirdlvld
2101 ynlmresrtq tyhfiktakg ritklvndfl kfslivqalk nnsswytelk klpevinvcn
2161 rfyhthncec qekffvqtly lqrlrdaeik lierltglmr fypegliysn ht

SEQ ID NO:37

Sudan Ebola virus polymerase

1 mmatqhtqyp darlsspivl dqcdlvtrac glyseyslnp klrtcrlpkh iyrlkydaiv
61 lrfisdvpva tipidyiapm linvladskn apleppclsf ldeivnytvq daaflnyymn
121 qiktqegvit dqlkqnirrv ihknrylsal ffwhdlsilt rrgrmnrgnv rstwfvtnev
181 vdilgygdyi fwkipiallp mnsanvphas tdwyqpnifk eaiqghthii svstaevlim
241 ckdlvtsrfn tlliaelarl edpvsadypl vddiqslyna gdyllsilgs egyqiikyle
301 plclakiqlc sqyterkgrf ltqmhlaviq tlrelllnrg lkksqlskir efhqlllrlr
361 stpqqlcelf siqkhwghpv lhsekaiqkv knhatvlkal rpiiifetyc vfkysvakhf
421 fdsqgtwysv isdrcltpgl nsyirrnqfp plpmikdllw efyhldhppl fstkiisdls
481 ifikdratav eqtcwdavfe pnvlgysppy rfntkrvpeq fleqedfsie svlqyaqelr
541 yllpqnrnfs fslkekelnv grtfgklpyl trnvqtlcea lladglakaf psnmmvvter
601 eqkesllhqa swhhtsddfg ehatvrgssf vtdlekynla fryeftapfi kycnqcygvr
661 nvfdwmhfli pqcymhvsdy ynpphnvtle nreyppegps ayrghlggie glqqklwtsi
721 scaqislvei ktgfklrsav mgdnqcitvl svfplesspn eqercaedna arvaaslakv
781 tsacgiflkp detfvhsgfi yfgpkqylng iqlpqslkta armaplsdai fddlqgtlas
841 igtafersis etrhilpsrv aaafhtyfsv rilqhhhlgf hkgsdlgqla inkpldfgti
901 alslavpqvl gglsflnpek clyrnlgdpv tsglfqlkhy lsmvgmsdif halvakspgn
961 csaidfvlnp gglnvpgsqd ltsflrqivr rsitlsarnk lintlfhasa dledelvckw
1021 llsstpvmsr faadifsrtp sgkrlqilgy legtrtllas kmisnnaetp ilerlrkitl
1081 qrwnlwfsyl dhcdsalmea iqpirctvdi aqilreyswa hilggrqlig atlpcipeqf
1141 qttwlkpyeq cvecsstnns spyvsvalkr nvvsawpdas rlgwtigdgi pyigsrtedk
1201 igqpaikprc psaalreaie ltsrltwvtq gsansdqlir pflearvnls vqeilqmtps
1261 hysgnivhry ndqysphsfm anrmsntatr lmvstntlge fsgggqaard sniifqnvin
1321 favalydirf rntctssiqy hrahihltdc ctrevpaqyl tytttlnldl skyrnneliy
1381 dseplrggln cnlsidsplm kgprlniied dlirlphlsg welaktvlqs iisdssnsst
1441 dpissgetrs ftthfltypk igllysfgal isfylgntil ctkkigltef lyylqnqihn
1501 lshrslrifk ptfrhssvms rlmdidpnfs iyiggtagdr glsdaarlfl riaistflsf
1561 veewvifrka niplwvvypl egqrpdppge flnrvksliv gieddknkgs ilsrseekcs
1621 snlvynckst asnffhasla ywrgrhrpkk tigatkatta phiilplgns drppgldlnq
1681 sndtfiptri kqivqgdsrn drttttrlpp qsrstptsat epptkiyegs ttyrgkstdt
1741 hldeghnake fpfnphrlvv pffkltkdge ysiepspees rsnikgllqh lrtmvdttiy
1801 crftgivssm hykldevlwe ynkfesavtl aegegsgall liqkygvkkl flntlatehs
1861 iesevisgyt tprmllsvmp rthrgelevi lnnsasqitd ithrdwfsnq knripndvdi
1921 itmdaetten ldrsrlyeav ytiicnhinp ktlkvvilkv flsdldgmcw innylapmfg
1981 sgylikpits sarssewylc lsnllstlrt tqhqtqancl hvvqcalqqq vqrgsywlsh
2041 ltkyttsrlh nsyiafgfps lekvlyhryn lvdsrngplv sitrhlallq teirelvidy
2101 nqlrqsrtqt yhfiktskgr itklvndylr felviralkn nstwhhelyl lpeligvchr
2161 fnhtrnctcs erflvqtlyl hrmsdaeikl mdrltslvnm fpegfrsssv

SEQ ID NO:34

Cote d/Ivoire Ebola virus polymerase

1 matqhtqypd arlsspivld qcdlvtracg lysayslnpq lkncrlpkhi yrlkydttvt
61 eflsdvpvat lpadflvptf lrtlsgngsc pidpkcsqfl eeivnytlqd irflnyylnr
121 agvhndhvdr dfgqkirnli cdnevlhqmf hwydlailar rgrlnrgnnr stwfasdnlv

181 dilgygdyif wkiplsllpv dtqglphaak dwyhesvfke aiqghthivs istadvlimc
241 kdiitcrfnt lliaavanle dsvhsdyplp etvsdlykag dylisllgse gykvikflep
301 lclakiqlcs nyterkgrfl tqmhlavnht leeltgsrel rpqqirkvre fhqmlinlka
361 tpqqlcelfs vqkhwghpvl hsekaiqkvk khatvikalr piiifetycv fkysiakhyf
421 dsqgtwysvt sdrcltpgls syikrnqfpp lpmikellwe fyhldhpplf stkvisdlsi
481 fikdratave ktcwdavfep nvlgynppnk fatkrvpeqf leqenfsies vlhyaqrley
541 llpeyrnfsf slkekelnig rafgklpypt rnvqtlceal ladglakafp snmmvvtere
601 qkesllhqas whhtsddfge natvrgssfv tdlekynlaf ryeftapfie ycnrcygvrn
661 lfnwmhytip qcyihvsdyy npphgvslen renppegpss yrghlggieg lqqklwtsis
721 caqislveik tgfklrsavm gdnqcitvls vfpletesse qelssednaa rvaaslakvt
781 sacgiflkpd etfvhsgfiy fgkkqylngv qlpqslktat riaplsdaif ddlqgtlasi
841 gtafersise trhvvpcrva aafhtffsvr ilqyhhlgfn kgtdlgqlsl skpldfgtit
901 lalavpqvlg glsflnpekc fyrnlgdpvt sglfqlktyl qmihmddlfl pliaknpgnc
961 saidfvlnps glnvpgsqdl tsflrqivrr titlsaknkl intlfhssad ledemvckwl
1021 lsstpvmsrf aadifsrtps gkrlqilgyl egtrtllask iinhntetpi ldrlrkitlq
1081 rwslwfsyld hcdqvladal tqitctvdla qilreytwah ilegrqliga tlpcileqln
1141 viwlkpyehc pkcaksanpk gepfvsiaik khvvsawpdq srlswtigdg ipyigsrted
1201 kigqpaikpk cpsaalreai eltsrltwvt qggansdllv kpfiearvnl svqeilqmtp
1261 shysgnivhr yndqysphsf manrmsnsat rlvvstntlg efsgggqsar dsniifqnvi
1321 nfavalfdlr frnvatssiq hhrahlhlsk cctrevpaqy lvytstlpld ltryrdneli
1381 yddnplrggl ncnlsfdnpl fkgqrlniie edlirlpyls gwelaktviq siisdsnnss
1441 tdpissgetr sftthfltyp kigllysfga lisyylgnti irtkkltlnn fiyylatqih
1501 nlphrslril kptlkhasvi srlisidshf siyiggtagd rglsdaarlf lrtaitvflq
1561 fvrkwiverk taiplwviyp legqspspin sflhhviall qhesshdhvc aaeahsrvet
1621 fdnlvymcks tasnffhasl aywrsrsknq dkremtkils ltqtekknsf gytahpesta
1681 vlgslqtsla pppsadeaty drknkvlkas rpgkysqntt kappnqtscr dvspnitgtd
1741 gcpsanegsn snnnnlvshr ivlpfftlsh nynerpsirk segtteivrl trqlraipdt
1801 tiycrftgiv ssmhykldev lwefdnfksa itlaegegsg allllqkykv etlffntlat
1861 ehsieaeiis gittprmllp imsrfhggqi kvtlnnsasq itditnpswl adqksripkq
1921 veiitmdaet teninrskly eavqqlivsh idpnalkvvv lkvflsdidg ilwlndnltp
1981 lfglgylikp itsspkssew ylclsnllst srrlphqsht tcmhviqtal qlqiqrssyw
2041 lshlvqyanh nlhldyinlg fpslervlyh rynlvdsqkg pltsivqhla hlqteirelv
2101 ndynqqrqsr tqtyhfikti kgritklvnd ylkffliiqa lkhnctwqee lralpdlisv
2161 ctrfyhtrnc scenrflvqt lylsrmqdse iklidrltgl lslcpngffr

SEQ ID NO:40

RECOMBINANT BIOLOGICALLY CONTAINED FILOVIRUS VACCINE

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of the filing date of U.S. application No. 62/715,673, filed Aug. 7, 2018, the disclosure of which is incorporated by reference herein.

STATEMENT OF GOVERNMENT RIGHTS

[0002] This invention was made with government support under AI109762 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND

[0003] *Ebolaviruses* cause hemorrhagic fevers in humans and nonhuman primates, with case fatality rates of 90% in some outbreaks (Sanchez et al., 2007). *Ebolaviruses* and the closely related *Marburgviruses* belong to the Filoviridae family (Feldman et al., 2004). Currently, there are no approved vaccines or antivirals for use against filoviruses, making biosafety level-4 (BSL-4) containment a mandatory requirement for work with these viruses. The lack of sufficient BSL-4 space, trained personnel, and the rigors of working in BSL-4 laboratories have severely hampered basic research with *Ebolaviruses* as well as the development of vaccines and large-scale screening for effective antiviral compounds. These limitations have prompted examination of various steps in the *Ebolavirus* viral life cycle in the absence of infectious virus: (i) replication and transcription were studied by use of reporter gene assays that are based on the expression of necessary viral components from plasmids (Boehmann et al., 2005; Groseth et al., 2005; Muhlberger et al., 1999; Modrof et al., 2003; Modrof et al., 2002); (ii) entry and fusion processes were assessed with pseudotyping assays that rely on the use of recombinant vesicular stomatitis or retroviruses (Yonezawa et al., 2005; Wool-Lewis et al., 1998; Takada et al., 1997; Marzi et al., 2006); and (iii) budding was examined using virus-like particles that are generated from viral proteins provided by protein expression plasmids (Jasenosky et al., 2001; Licata et al., 2004; Noda et al., 2002; McCarthy et al., 2006; Johnson et al., 2006). However, several recent findings suggest that data obtained with these artificial systems may not always be reproducible with live, authentic *Ebolavirus* (Neumann et al., 2005). Thus, biologically contained *Ebolaviruses* that resemble wild-type virus but can be handled outside BSL-4 containment are clearly needed.

SUMMARY

[0004] The invention provides a vaccine comprising an effective amount of a recombinant negative-sense, single stranded RNA virus, the genome of which contains a deletion of viral sequences corresponding to those for a nonstructural or nonglycosylated viral protein that is essential in trans for viral replication, and in one embodiment, one or more adjuvants, or in one embodiment, one or more insertions of a nucleotide sequence encoding one or more heterologous gene products, or in one embodiment, one or more adjuvants and one or more insertions of a nucleotide sequence encoding one or more heterologous gene products, wherein the insertions may be in coding or non-coding sequences. In one embodiment, the heterologous gene product is from a Zaïre, Sudan, Côte d'Ivoire, Bundibugyo, Reston, or *Marburg* filovirus, a glycoprotein of one or more of those filoviruses. In one embodiment, the insertions may replace coding sequences, e.g., glycoprotein coding sequences, or a portion thereof, or may replace non-coding sequences. In one embodiment, the deletion is effective to inhibit or prevent viral replication upon infection of a cell with the recombinant negative-sense, single stranded RNA virus. For example, the deletion of viral sequences corresponding to those for a nonstructural or nonglycosylated viral protein that is essential in trans for viral replication may be effective to prevent expression of a functional nonstructural or nonglycosylated protein upon infection of a cell with the recombinant negative-sense, single stranded RNA virus. In one embodiment, the deletion of viral sequences corresponding to those for a nonstructural or nonglycosylated viral protein that is essential in trans for viral replication may be in filovirus sequences for a viral protein corresponding to *Ebola virus* VP30 protein, In one embodiment, the genome of the recombinant, biologically contained filovirus comprises heterologous sequences, for instance, positioned within the deletion in VP30 related sequences. Any of the deletions in viral sequences of a negative-sense, single stranded RNA virus may include a deletion of 1 or more nucleotides, e.g., a deletion of at least 0.1%, 1%, 5%, 10%, 50%, 60%, 70%, 80%, 90%, or any integer in between, and up to 100% of the viral sequences corresponding to those for a nonstructural, glycosylated or nonglycosylated viral protein. The deletion of viral sequences corresponding to those for a nonstructural or nonglycosylated viral protein that is essential in trans for viral replication is one that is stable over multiple passages and is readily detectable, e.g., by RT-PCR. In one embodiment, the genome of the recombinant virus has a deletion in viral sequences for two or more nonstructural or nonglycosylated proteins, for example, a deletion in sequences for viral proteins that are not contiguous with each other, such as sequences for a viral protein corresponding to *Ebola virus* VP30 protein and for a viral protein corresponding to *Ebola virus* GP protein. In one embodiment, where the genome of the recombinant virus has a deletion in viral sequences for a nonstructural, glycosylated or nonglycosylated protein, at least a portion of the deleted viral sequences may be replaced with a nucleotide sequence encoding an antigen that is expressed in the recombinant filovirus which, when administered to a mammal, is prophylactic or therapeutic. In one embodiment, where the genome of the recombinant virus has a deletion in viral sequences for two or more proteins that are nonstructural, glycosylated or nonglycosylated proteins, at least a portion of one of the deleted viral sequences may be replaced with a nucleotide sequence encoding an antigen that is expressed in the recombinant filovirus which, when administered to a mammal, is prophylactic or therapeutic. The vaccine of the invention may provide for subtype cross protection, for filovirus cross protection and optionally as a bi- or multi-valent vaccine for pathogens other than filovirus.

[0005] As shown hereinbelow, incorporating an adjuvant into the vaccine provided unexpected results. Moreover, not all adjuvants were effective, e.g., alum did not enhance the efficacy of the vaccine virus of the invention relative to a control (no adjuvant). In one embodiment, a monovalent recombinant filovirus vaccine comprises one or more adjuvants and a recombinant filovirus, the expression of the

genome results in a virus having a homologous glycoprotein, e.g., a Zaire genome expresses a Zaire glycoprotein. In one embodiment, a monovalent recombinant filovirus vaccine comprises one or more adjuvants and a recombinant filovirus, the expression of the genome results in a virus having a heterologous glycoprotein, e.g., a Zaire genome expresses a *Marburg virus,* Sudan *Ebolavirus,* or Bundibugyo *Ebolavirus* glycoprotein, e.g., inserted into ORF 4 (to replace the parent glycoprotein). In one embodiment, a monovalent recombinant filovirus vaccine comprises one or more adjuvants and a recombinant filovirus, the expression of the genome results in a virus having a heterologous glycoprotein, e.g., a Zaire genome expresses a *Marburg virus.* Sudan *Ebolavirus,* or Bundibugyo *Ebolavirus* glycoprotein, e.g., inserted into sequences corresponding to *Ebola virus* VP30 (ORF5) or the resulting deletion of ORF5 or a portion thereof, e.g., two different glycoproteins are expressed.

[0006] In one embodiment, a vaccine comprising an effective amount of a recombinant filovirus and one or more adjuvants is provided. The genome of the recombinant filovirus contains a deletion of one or more nucleotides in a polynucleotide sequence for a viral protein corresponding to *Ebola virus* VP30, and the deletion is effective to prevent expression of a functional viral protein corresponding to *Ebola virus* VP30 upon infection of a cell with the recombinant filovirus. In one embodiment, at least 90% of sequences corresponding to VP30 sequences in the viral genome of the virus are deleted. In one embodiment, the genome further comprises a nucleotide sequence encoding a prophylactic or therapeutic heterologous gene product. In one embodiment, the nucleotide sequence is inserted within 500 nucleotides of the deletion site or at the site of the deletion. In one embodiment, the nucleotide sequence is inserted into the filovirus genome at a site other than the site of the deletion in the polynucleotide. In one embodiment, the nucleotide sequence is inserted between NP coding sequences and VP35 coding sequences in the filovirus genome. In one embodiment, the nucleotide sequence replaces GP/sGP sequences or a portion thereof. In one embodiment, the nucleotide sequence is inserted into GP/sGP coding sequences. In one embodiment, the heterologous gene product comprises a heterologous filovirus glycoprotein. In one embodiment, the filovirus glycoprotein comprises a *Marburg virus, Ebola virus,* Sudan virus, Tai Forest virus, Reston virus, or Bundibugyo virus glycoprotein. In one embodiment, the recombinant filovirus genome is a recombinant *Ebola virus* genome. In one embodiment, the vaccine of further comprises a pharmaceutically acceptable carrier.

[0007] In one embodiment, the adjuvant comprises lipopolysaccharide. In one embodiment, the lipopolysaccharide comprises monophosphoryl lipid A. In one embodiment, the adjuvant comprises squalene. In one embodiment, the adjuvant comprises an extract of *Quillaja saponaria.* In one embodiment, the adjuvant comprises saponin. In one embodiment, the recombinant filovirus in the vaccine is inactivated. A method to immunize a mammal using a composition having the recombinant filovirus is also provided. In one embodiment, the mammal is a human. In one embodiment, two doses of the composition are administered. In one embodiment, a single dose is administered. In one embodiment, three doses of the composition are administered. In one embodiment, the recombinant filovirus is inactivated, e.g., using heat, one or more chemicals, e.g., formaldehyde, formalin, beta-propiolactone, diethylpyrocarbonate, an oxidizing agent such as hydrogen peroxide, 2,2'-dithiodipyridine, binary ethylene imine, glutaraldehyde or radiation, e.g., gamma or UV.

[0008] Since most areas in Africa experience several specific endemic or recurring diseases, and the combinations vary among regions, the disclosure provide for bi-/multivalent vaccines to address combinations of diseases that impact particular areas. Monovalent vaccines may be particularly useful in response to any outbreaks that don't correspond well to other vaccines. Multivalent vaccines may be based on the addition of exogenous sequences into any of several positions in the filovirus genome including but not limited to: 1) an artificial transcriptional unit between open reading frame (ORF) 1 (e.g., NP) and ORF 2 (e.g., VP35), 2) ORF 4 (e.g., Zaire glycoprotein gene), and 3) ORF 5 (e.g., VP30 gene). In one embodiment, a bivalent vaccine virus may express a one or more nonglycosylated proteins, one or more glycosylated proteins, or at least one nonglycosylated protein and at least one glycosylated protein from, for example, Zaire *Ebolavirus* and *Marburg virus, Ebola* and *Marburg* viruses, a filovirus and Lassa virus, or a filovirus and *Plasmodium* (malaria).

[0009] Thus, in one embodiment, a recombinant filovirus, wherein the genome of the recombinant filovirus contains a first deletion of one or more nucleotides in a polynucleotide sequence for a viral protein corresponding to *Ebola virus* VP30 which first deletion is effective to prevent expression of a functional viral protein corresponding to *Ebola virus* VP30 upon infection of a cell with the recombinant filovirus, and the genome encodes one or more filovirus glycoproteins. The genome may contain a mutation in a region that is flanked by NP coding sequences and VP35 coding sequences, a mutation in GP/sGP coding sequences, and/or an insertion within 500 nucleotides of the first deletion site or at the first deletion site, or a combination thereof, and the genome encodes one or more filovirus glycoproteins. The mutation in the region that is flanked by NP coding sequences and VP35 coding sequences comprises an insertion of a nucleotide sequence encoding a prophylactic or therapeutic heterologous gene product and optionally also a deletion of one or more nucleotides in the region that flanks the NP coding sequences and VP35 coding sequences. The mutation in the GP/sGP coding sequences comprises an insertion of a nucleotide sequence encoding a prophylactic or therapeutic heterologous gene product and optionally also a deletion of one or more nucleotides in the GP/sGP coding sequences. The insertion that is within 500 nucleotides of or at the first deletion site encodes a prophylactic or therapeutic heterologous gene product. In one embodiment, the recombinant filovirus is inactivated, e.g., using heat, one or more chemicals, e.g., formaldehyde, formalin, beta-propiolactone, diethylpyrocarbonate, an oxidizing agent such as hydrogen peroxide, 2,2'-dithiodipyridine, binary ethylene imine, glutaraldehyde or radiation, e.g., gamma or UV.

[0010] Further provided is a multivalent vaccine comprising an effective amount of a recombinant filovirus, wherein the genome of the recombinant filovirus contains a first deletion in one or more nucleotides for a polynucleotide sequence for a viral protein corresponding to *Ebola virus* VP30 which deletion is effective to prevent expression of a functional viral protein corresponding to *Ebola virus* VP30 upon infection of a cell with the recombinant filovirus, and

wherein the genome encodes one or more filovirus glycoproteins. The genome may contain a mutation in a region that is flanked by NP coding sequences and VP35 coding sequences, a mutation in GP/sGP coding sequences, and/or an insertion within 500 nucleotides of the first deletion site or at the first deletion site, or a combination thereof. The mutation in the region that is flanked by NP coding sequences and VP35 coding sequences comprises an insertion of a nucleotide sequence encoding a prophylactic or therapeutic heterologous gene product and optionally also a deletion of one or more nucleotides in the region that flanks the NP coding sequences and VP35 coding sequences. The mutation in the GP/sGP coding sequences comprises an insertion of a nucleotide sequence encoding a prophylactic or therapeutic heterologous gene product and optionally also a deletion of one or more nucleotides in the GP/sGP coding sequences. The insertion that is within 500 nucleotides, e.g., within at least 1000 nucleotides, of or at the first deletion site encodes a prophylactic or therapeutic heterologous gene product. In one embodiment, one of the filovirus glycoproteins encoded by the genome comprises a homologous filovirus glycoprotein. In one embodiment, one of the filovirus glycoproteins encoded by the genome comprises a heterologous filovirus glycoprotein. In one embodiment, the prophylactic or therapeutic heterologous gene product is not a glycoprotein. In one embodiment, the prophylactic or therapeutic heterologous gene In one embodiment, product comprises a glycoprotein. In one embodiment, the vaccine further comprises an adjuvant. In one embodiment, the adjuvant comprises lipopolysaccharide. In one embodiment, the adjuvant comprises squalene. In one embodiment, the adjuvant comprises an extract of *Quillaja saponaria*. In one embodiment, the adjuvant comprises saponin. In one embodiment, the vaccine further comprises a pharmaceutically acceptable carrier. In one embodiment, the recombinant filovirus in the vaccine is inactivated. Further provided is a method to immunize a mammal, e.g., a human, by administering to the mammal an effective amount of the vaccine. For example, a human in contact with filovirus infected individuals or inadvertently exposed to filovirus, e.g., in a laboratory, may be administered the recombinant infectious, biologically contained virus of the invention in an amount effective to inhibit or substantially eliminate filovirus replication in the human.

[0011] To prepare such viruses, a reverse genetics systems for negative-sense RNA viruses was exploited to generate *Ebolaviruses* that lack a substantial portion of the VP30 gene (which encodes an essential transcription factor), termed *Ebola*ΔVP30 virus, lack a substantial portion of the L gene, or lack a substantial portion of both genes. *Ebola*ΔVP30 viruses were maintained, genetically stable, and biologically confined to a cell line expressing VP30. Hence, the *Ebola*ΔVP30 virus fulfills several criteria of a vaccine virus: it can be grown to reasonably high titers in helper cells, is genetically stable (as determined by sequence analysis after seven serial passages in VP30-expressing Vero cells), and is safe. Moreover, the resultant viruses resemble wild-type virus in their life cycle, their morphology, and their growth properties, but could be handled in a non-BSL-4 laboratory, opening new opportunities for study of the *Ebolavirus* life cycle and for the identification of effective antiviral compounds.

[0012] Other negative-sense, single stranded RNA viruses may likewise be manipulated, e.g., the genome of Nipah virus, Hendravirus, Henipavirus, and the like, may be manipulated to mutate or delete sequences corresponding to those for a nonstructural or nonglycoslyated viral protein that is required for viral replication. Thus, genomes of viruses in the following families may be manipulated to provide for an infectious, biologically contained virus that resembles wild-type virus in its life cycle, morphology, and growth properties, can be grown to reasonably high titers in helper cells, is genetically stable, and is safe: Bornaviridae, Rhabdoviridae, Filoviridae (genera *Marburgvirus* and *Ebolavirus*), Paramyxoviridae, Avulavirus, Henipavirus, Morbillivirus, Respirovirus, or Rubulavirus.

[0013] The disclosure also provides a method to prepare an infectious, biologically contained negative-sense, single stranded RNA virus, e.g., filovirus. In one embodiment, the method includes providing a host cell, e.g., a Vero cell, having a plurality of viral vectors which when expressed (stably or transiently) are effective to yield infectious, biologically contained negative-sense, single stranded RNA virus. In one embodiment, the plurality of vectors includes a vector for vRNA production comprising a promoter operably linked to a virus DNA which contains a deletion of sequences for a viral gene corresponding to *Ebola virus* VP30 which deletion is effective to prevent expression of a functional viral protein corresponding to *Ebola virus* VP30, linked to a transcription termination sequence, and an insertion of heterologous sequences as discussed above. The host cell also includes a vector for mRNA production comprising a promoter operably linked to a DNA segment encoding a viral polymerase, a vector for mRNA production comprising a promoter operably linked to a DNA segment encoding viral nucleoprotein, a vector for mRNA production comprising a promoter operably linked to a DNA segment encoding one or more other viral proteins which along with the viral polymerase and nucleoprotein, are viral proteins needed for viral replication, and a vector comprising a promoter operably linked to a DNA encoding a RNA polymerase that is heterologous to the host cell. The heterologous RNA polymerase is selected to promote transcription of the viral DNA which contains the deletion. In one embodiment, the vector for vRNA includes a 17 polymerase promoter and a ribozyme sequence capable of cleaving a transcript to yield a vRNA-like 3' end. Then infectious, biologically contained virus is isolated from the cell. In one embodiment, the host cell is transiently transfected with the plurality of vectors and virus collected within 1, 2, 3, and up to 7 days post-transfection. In one embodiment, the host cell is one that is approved for vaccine production. In one embodiment, additional heterologous sequences are included in the vRNA vector or in mRNA vectors subsequently introduced to the host cell, and/or are introduced to the host cell via a mRNA vector. In one embodiment, the additional heterologous sequences are for an immunogenic polypeptide or peptide of a pathogen, a tumor antigen, or a therapeutic protein.

[0014] In one embodiment, a method to prepare a multivalent infectious, biologically contained filovirus is provided. The method includes providing a host cell comprising a plurality of filovirus vectors which, when expressed in the host cell, are effective to yield infectious, biologically contained filovirus, wherein the plurality of vectors includes a vector for vRNA production comprising a promoter operably linked to a filovirus DNA which contains a deletion in sequences for a functional viral protein corresponding to

Ebola virus VP30, which deletion is effective to prevent expression of the functional viral protein linked to a transcription termination sequence, and other sequences as disclosed herein above, a vector for mRNA production comprising a promoter operably linked to a DNA segment encoding a filovirus polymerase, a vector for mRNA production comprising a promoter operably linked to a DNA segment encoding a filovirus nucleoprotein, a vector for mRNA production comprising a promoter operably linked to a DNA segment encoding a filovirus protein corresponding to *Ebola virus* VP30, a vector for mRNA production comprising a promoter operably linked to a DNA segment encoding a filovirus protein corresponding to *Ebola virus* VP35, and a vector for mRNA production comprising a promoter operably linked to a DNA encoding a RNA polymerase that is heterologous to the host cell, wherein the heterologous RNA polymerase promotes transcription of vRNA from the filovirus DNA which contains the deletion; and isolating infectious, biologically contained filovirus from the host cell. In one embodiment, the cells are mammalian cells. In one embodiment, the cells are primate cells. In one embodiment, the cells are Vero cells. In one embodiment, the heterologous RNA polymerase is a T3, T7, or SP6 polymerase. In one embodiment, the gene product sequences for an immunogenic polypeptide or peptide of a pathogen, a tumor antigen, or a therapeutic protein. In one embodiment, each vector encoding a filovirus protein is on a separate plasmid.

[0015] Further provided is a method of manufacturing, e.g., large scale manufacturing, recombinant filovirus, e.g., for vaccine production. The method includes culturing mammalian cells expressing a recombinant filovirus genome in serum free medium in a cell culture system so as to result in progeny recombinant biologically contained filovirus. The genome of the recombinant filovirus contains a deletion of one or more nucleotides in a polynucleotide sequence for a viral protein corresponding to *Ebola virus* VP30, and wherein the deletion is effective to prevent expression of a functional viral protein corresponding to *Ebola virus* VP30 upon infection of a cell with the recombinant filovirus. The mammalian cells express a viral protein corresponding to *Ebola virus* VP30. In one embodiment, the serum free media comprises non-animal proteins or peptides, e.g., plant proteins or peptides, an iron chelator, e.g., EDTA, ferric nitrate, ferrous sulfate, or transferrin, or a combination thereof. The supernatant from the mammalian cells having the progeny is collected and contacted with a DNase and a virus inactivating agent, e.g., beta-propriolactone, heat, formaldehyde, gamma radiation, or hydroxylamine, thereby providing an inactivated viral preparation. The inactivated viral preparation is then purified, concentrated, desalted and/or fractionated from other molecules, e.g., via filtration, optionally under conditions that do not result in precipitation, e.g., precipitation visible to the naked eye, of the inactivated viral particles.

[0016] In one embodiment, the mammalian cells are Vero cells. In one embodiment, the collected supernatant is subject to separation, e.g., filtration, before contact with the DNase or the viral inactivating agent. In one embodiment, the collected supernatant is subjected to filtration with a 0.5 to 5 micron filter or a 1 to 5 micron filter. In one embodiment, the collected supernatant is subjected to filtration with 0.01 to 1 micron filter or a 0.05 to 0.25 micron filter. In one embodiment, the inactivated viral preparation is subjected to filtration through a 0.01 to 1 micron filter or a 0.05 to 0.25 micron filter. In one embodiment, the inactivated viral preparation is combined with one or more adjuvants.

BRIEF DESCRIPTION OF THE FIGURES

[0017] FIG. **1**. Schematic diagram of *Ebola*ΔVP30 constructs. (Top row) Schematic diagram of the *Ebolavirus* genome flanked by the leader sequence (I) and the trailer sequence (t) in positive-sense orientation. Two unique restriction sites for Sail and Sad (positions 6180 and 10942 of the viral antigenome, respectively) allowed the subcloning of a fragment that spans the VP30 gene. The subgenomic fragment was then used to replace the VP30 gene with genes encoding neomycin (neo) or enhanced green fluorescence protein (eGFP), respectively. Using the unique restriction sites, the altered subgenomic fragments were cloned back into the full-length *Ebolavirus* cDNA construct.

[0018] FIG. **2**. Representative filovirus sequences (Accession numbers NC006432, NC004161, AY769362, AY142960, AF522874, AF499101, L11365, NC001608, DQ447652, DQ447649, AB050936, NC002549, NC001608, AF086833 and AF272001, the disclosures of which are incorporated by reference herein: SEQ ID Nos. 1-15 and 18-40).

DETAILED DESCRIPTION

Definitions

[0019] A “vector” or “construct” (sometimes referred to as gene delivery or gene transfer “vehicle”) refers to a macromolecule or complex of molecules comprising a polynucleotide or virus to be delivered to a host cell, either in vitro or in vivo. The polynucleotide or virus to be delivered may comprise a coding sequence of interest for gene therapy. Vectors include, for example, viral vectors (such as filoviruses, adenoviruses, adeno-associated viruses (AAV), lentiviruses, herpesvirus and retroviruses), liposomes and other lipid-containing complexes, and other macromolecular complexes capable of mediating delivery of a polynucleotide to a host cell. Vectors can also comprise other components or functionalities that further modulate gene delivery and/or gene expression, or that otherwise provide beneficial properties to the targeted cells. Such other components include, for example, components that influence binding or targeting to cells (including components that mediate cell-type or tissue-specific binding); components that influence uptake of the vector nucleic acid by the cell; components that influence localization of the polynucleotide within the cell after uptake (such as agents mediating nuclear localization); and components that influence expression of the polynucleotide. Such components also might include markers, such as detectable and/or selectable markers that can be used to detect or select for cells that have taken up and are expressing the nucleic acid delivered by the vector. Such components can be provided as a natural feature of the vector (such as the use of certain viral vectors which have components or functionalities mediating binding and uptake), or vectors can be modified to provide such functionalities. A large variety of such vectors are known in the art and are generally available. When a vector is maintained in a host cell, the vector can either be stably replicated by the cells during

mitosis as an autonomous structure, incorporated within the genome of the host cell, or maintained in the host cell's nucleus or cytoplasm.

[0020] A "recombinant viral vector" refers to a viral vector comprising one or more modifications, including deletions, insertions and/or heterologous genes or sequences. Since many viral vectors exhibit size constraints associated with packaging, the heterologous genes or sequences are typically introduced by replacing one or more portions of the viral genome. Such viruses may become replication-defective (biologically contained), requiring the deleted function (s) to be provided in trans during viral replication and encapsidation (by using, e.g., a helper virus or a packaging cell line carrying genes necessary for replication and/or encapsidation). Modified viral vectors in which a polynucleotide to be delivered is carried on the outside of the viral particle have also been described.

[0021] "Gene delivery," "gene transfer," and the like as used herein, are terms referring to the introduction of an exogenous polynucleotide (sometimes referred to as a "transgene") into a host cell, irrespective of the method used for the introduction. Such methods include a variety of well-known techniques such as vector-mediated gene transfer (by, e.g., viral infection/transfection, or various other protein-based or lipid-based gene delivery complexes) as well as techniques facilitating the delivery of "naked" polynucleotides (such as electroporation, "gene gun" delivery and various other techniques used for the introduction of polynucleotides). The introduced polynucleotide may be stably or transiently maintained in the host cell. Stable maintenance typically requires that the introduced polynucleotide either contains an origin of replication compatible with the host cell or integrates into a replicon of the host cell such as an extrachromosomal replicon (e.g., a plasmid) or a nuclear or mitochondrial chromosome. A number of vectors are known to be capable of mediating transfer of genes to mammalian cells, as is known in the art.

[0022] By "transgene" is meant any piece of a nucleic acid molecule (for example, DNA) which is inserted by artifice into a cell either transiently or permanently, and becomes part of the organism if integrated into the genome or maintained extrachromosomally. Such a transgene may include at least a portion of an open reading frame of a gene which is partly or entirely heterologous (i.e., foreign) to the transgenic organism, or may represent at least a portion of an open reading frame of a gene homologous to an endogenous gene of the organism, which portion optionally encodes a polypeptide with substantially the same activity as the corresponding full-length polypeptide or at least one activity of the corresponding full-length polypeptide.

[0023] By "transgenic cell" is meant a cell containing a transgene. For example, a cell stably or transiently transformed with a vector containing an expression cassette is a transgenic cell that can be used to produce a population of cells having altered phenotypic characteristics. A "recombinant cell" is one which has been genetically modified, e.g., by insertion, deletion or replacement of sequences in a nonrecombinant cell by genetic engineering.

[0024] The term "wild-type" or "native" refers to a gene or gene product that has the characteristics of that gene or gene product when isolated from a naturally occurring source. A wild-type gene is that which is most frequently observed in a population and is thus arbitrarily designated the "normal" or "wild-type" form of the gene. In contrast, the term "modified" or "mutant" refers to a gene or gene product that displays modifications in sequence and or functional properties (i.e., altered characteristics) when compared to the wild-type gene or gene product. It is noted that naturally-occurring mutants can be isolated; these are identified by the fact that they have altered characteristics when compared to the wild-type gene or gene product.

[0025] The term "transduction" denotes the delivery of a polynucleotide to a recipient cell either in vivo or in vitro, via a viral vector and preferably via a replication-defective viral vector.

[0026] The term "heterologous" as it relates to nucleic acid sequences such as gene sequences encoding a protein and control sequences, denotes sequences that are not normally joined together, and/or are not normally associated with a particular cell, e.g., are from different sources (for instance, sequences from a virus are heterologous to sequences in the genome of an uninfected cell). Thus, a "heterologous" region of a nucleic acid construct or a vector is a segment of nucleic acid within or attached to another nucleic acid molecule that is not found in association with the other molecule in nature. For example, a heterologous region of a nucleic acid construct could include a coding sequence flanked by sequences not found in association with the coding sequence in nature, i.e., a heterologous promoter. Another example of a heterologous coding sequence is a construct where the coding sequence itself is not found in nature (e.g., synthetic sequences having codons different from the native gene). Similarly, a cell transformed with a construct which is not normally present in the cell would be considered heterologous for purposes of this invention.

[0027] By "DNA" is meant a polymeric form of deoxyribonucleotides (adenine, guanine, thymine, or cytosine) in double-stranded or single-stranded form found, inter alia, in linear DNA molecules (e.g., restriction fragments), viruses, plasmids, and chromosomes. In discussing the structure of particular DNA molecules, sequences may be described herein according to the normal convention of giving only the sequence in the 5' to 3' direction along the nontranscribed strand of DNA (i.e., the strand having the sequence complementary to the mRNA). The term captures molecules that include the four bases adenine, guanine, thymine, or cytosine, as well as molecules that include base analogues which are known in the art.

[0028] As used herein, the terms "complementary" or "complementarity" are used in reference to polynucleotides (i.e., a sequence of nucleotides) related by the base pairing rules. For example, the sequence "A-G-T," is complementary to the sequence "T-C-A." Complementarity may be "partial," in which only some of the nucleic acids' bases are matched according to the base pairing rules. Or, there may be "complete" or "total" complementarity between the nucleic acids. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of hybridization between nucleic acid strands. This is of particular importance in amplification reactions, as well as detection methods that depend upon binding between nucleic acids.

[0029] DNA molecules are said to have "5' ends" and "3' ends" because mononucleotides are reacted to make oligonucleotides or polynucleotides in a manner such that the 5' phosphate of one mononucleotide pentose ring is attached to the 3' oxygen of its neighbor in one direction via a phosphodiester linkage. Therefore, an end of an oligonucleotide

or polynucleotide is referred to as the "5' end" if its 5' phosphate is not linked to the 3' oxygen of a mononucleotide pentose ring and as the "3' end" if its 3' oxygen is not linked to a 5' phosphate of a subsequent mononucleotide pentose ring. As used herein, a nucleic acid sequence, even if internal to a larger oligonucleotide or polynucleotide, also may be said to have 5 and 3' ends. In either a linear or circular DNA molecule, discrete elements are referred to as being "upstream" or 5' of the "downstream" or 3' elements. This terminology reflects the fact that transcription proceeds in a 5' to 3' fashion along the DNA strand. The promoter and enhancer elements that direct transcription of a linked gene are generally located 5' or upstream of the coding region. However, enhancer elements can exert their effect even when located 3' of the promoter element and the coding region. Transcription termination and polyadenylation signals are located 3' or downstream of the coding region.

[0030] A "gene," "polynucleotide," "coding region," "sequence," "segment, " "fragment" or "transgene" which "encodes" a particular protein, is a nucleic acid molecule which is transcribed and optionally also translated into a gene product, e.g., a polypeptide, in vitro or in vivo when placed under the control of appropriate regulatory sequences. The coding region may be present in either a cDNA, genomic DNA, or RNA form. When present in a DNA form, the nucleic acid molecule may be single-stranded (i.e., the sense strand) or double-stranded. The boundaries of a coding region are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxy) terminus. A gene can include, but is not limited to, cDNA from prokaryotic or eukaryotic mRNA, genomic DNA sequences from prokaryotic or eukaryotic DNA, and synthetic DNA sequences. A transcription termination sequence will usually be located 3' to the gene sequence.

[0031] The term "control elements" refers collectively to promoter regions, polyadenylation signals, transcription termination sequences, upstream regulatory domains, origins of replication, internal ribosome entry sites ("IRES"), enhancers, splice junctions, and the like, which collectively provide for the replication, transcription, post-transcriptional processing and translation of a coding sequence in a recipient cell. Not all of these control elements need always be present so long as the selected coding sequence is capable of being replicated, transcribed and translated in an appropriate host cell.

[0032] The term "promoter" is used herein in its ordinary sense to refer to a nucleotide region comprising a DNA regulatory sequence, wherein the regulatory sequence is derived from a gene which is capable of binding RNA polymerase and initiating transcription of a downstream (3' direction) coding sequence.

[0033] By "enhancer" is meant a nucleic acid sequence that, when positioned proximate to a promoter, confers increased transcription activity relative to the transcription activity resulting from the promoter in the absence of the enhancer domain.

[0034] By "operably linked" with reference to nucleic acid molecules is meant that two or more nucleic acid molecules (e.g., a nucleic acid molecule to be transcribed, a promoter, and an enhancer element) are connected in such a way as to permit transcription of the nucleic acid molecule. "Operably linked" with reference to peptide and/or polypeptide molecules is meant that two or more peptide and/or polypeptide molecules are connected in such a way as to yield a single polypeptide chain, i.e., a fusion polypeptide, having at least one property of each peptide and/or polypeptide component of the fusion. The fusion polypeptide is preferably chimeric, i.e., composed of heterologous molecules.

[0035] "Homology" refers to the percent of identity between two polynucleotides or two polypeptides. The correspondence between one sequence and to another can be determined by techniques known in the art. For example, homology can be determined by a direct comparison of the sequence information between two polypeptide molecules by aligning the sequence information and using readily available computer programs. Alternatively, homology can be determined by hybridization of polynucleotides under conditions which form stable duplexes between homologous regions, followed by digestion with single strand-specific nuclease(s), and size determination of the digested fragments. Two DNA, or two polypeptide, sequences are "substantially homologous" to each other when at least about 80%, preferably at least about 90%, and most preferably at least about 95% of the nucleotides, or amino acids, respectively match over a defined length of the molecules, as determined using the methods above.

[0036] By "mammal" is meant any member of the class Mammalia including, without limitation, humans and non-human primates such as chimpanzees and other apes and monkey species; farm animals such as cattle, sheep, pigs, goats and horses; domestic mammals such as dogs and cats; laboratory animals including rodents such as mice, rats, rabbits and guinea pigs, and the like.

[0037] By "derived from" is meant that a nucleic acid molecule was either made or designed from a parent nucleic acid molecule, the derivative retaining substantially the same functional features of the parent nucleic acid molecule, e.g., encoding a gene product with substantially the same activity as the gene product encoded by the parent nucleic acid molecule from which it was made or designed.

[0038] By "expression construct" or "expression cassette" is meant a nucleic acid molecule that is capable of directing transcription. An expression construct includes, at the least, a promoter. Additional elements, such as an enhancer, and/or a transcription termination signal, may also be included.

[0039] The term "exogenous," when used in relation to a protein, gene, nucleic acid, or polynucleotide in a cell or organism refers to a protein, gene, nucleic acid, or polynucleotide which has been introduced into the cell or organism by artificial or natural means. An exogenous nucleic acid may be from a different organism or cell, or it may be one or more additional copies of a nucleic acid which occurs naturally within the organism or cell. By way of a non-limiting example, an exogenous nucleic acid is in a chromosomal location different from that of natural cells, or is otherwise flanked by a different nucleic acid sequence than that found in nature.

[0040] The term "isolated" when used in relation to a nucleic acid, peptide, polypeptide or virus refers to a nucleic acid sequence, peptide, polypeptide or virus that is identified and separated from at least one contaminant nucleic acid, polypeptide or other biological component with which it is ordinarily associated in its natural source, e.g., so that it is not associated with in vivo substances, or is substantially purified from in vitro substances. Isolated nucleic acid, peptide, polypeptide or virus is present in a form or setting that is different from that in which it is found in nature. For

example, a given DNA sequence (e.g., a gene) is found on the host cell chromosome in proximity to neighboring genes; RNA sequences, such as a specific mRNA sequence encoding a specific protein, are found in the cell as a mixture with numerous other mRNAs that encode a multitude of proteins. The isolated nucleic acid molecule may be present in single-stranded or double-stranded form. When an isolated nucleic acid molecule is to be utilized to express a protein, the molecule will contain at a minimum the sense or coding strand (i.e., the molecule may single-stranded), but may contain both the sense and anti-sense strands (i.e., the molecule may be double-stranded).

[0041] As used herein, the term "recombinant nucleic acid" or "recombinant DNA sequence, molecule or segment" refers to a nucleic acid, e.g., to DNA, that has been derived or isolated from a source, that may be subsequently chemically altered in vitro, and includes, but is not limited to, a sequence that is naturally occurring, is not naturally occurring, or corresponds to naturally occurring sequences that are not positioned as they would be positioned in the native genome. An example of DNA "derived" from a source, would be a DNA sequence that is identified as a useful fragment, and which is then chemically synthesized in essentially pure form. An example of such DNA "isolated" from a source would be a useful DNA sequence that is excised or removed from said source by chemical means, e.g., by the use of restriction endonucleases, so that it can be further manipulated, e.g., amplified, for use in the invention, by the methodology of genetic engineering.

[0042] The term "recombinant protein" or "recombinant polypeptide" as used herein refers to a protein molecule that is expressed from a recombinant DNA molecule.

[0043] The term "peptide", "polypeptide" and protein" are used interchangeably herein unless otherwise distinguished.

[0044] The term "sequence homology" means the proportion of base matches between two nucleic acid sequences or the proportion amino acid matches between two amino add sequences. When sequence homology is expressed as a percentage, e.g., 50%, the percentage denotes the proportion of matches over the length of a selected sequence that is compared to some other sequence. Gaps (in either of the two sequences) are permitted to maximize matching; gap lengths of 15 bases or less are usually used, 6 bases or less are preferred with 2 bases or less more preferred. When using oligonucleotides as probes or treatments, the sequence homology between the target nucleic acid and the oligonucleotide sequence is generally not less than 17 target base matches out of 20 possible oligonucleotide base pair matches (85%); preferably not less than 9 matches out of 10 possible base pair matches (90%), and more preferably not less than 19 matches out of 20 possible base pair matches (95%).

[0045] The term "selectively hybridize" means to detectably and specifically bind. Polynucleotides, oligonucleotides and fragments of the invention selectively hybridize to nucleic acid strands under hybridization and wash conditions that minimize appreciable amounts of detectable binding to nonspecific nucleic acids. High stringency conditions can be used to achieve selective hybridization conditions as known in the art and discussed herein. Generally, the nucleic acid sequence homology between the polynucleotides, oligonucleotides, and fragments of the invention and a nucleic acid sequence of interest is at least 65%, and more typically with preferably increasing homologies of at least about 70%, about 90%, about 95%, about 98%, and 100%.

[0046] Two amino acid sequences are homologous if there is a partial or complete identity between their sequences. For example, 85% homology means that 85% of the amino acids are identical when the two sequences are aligned for maximum matching. Gaps (in either of the two sequences being matched) are allowed in maximizing matching; gap lengths of 5 or less are preferred with 2 or less being more preferred. Alternatively and preferably, two protein sequences (or polypeptide sequences derived from them of at least 30 amino acids in length) are homologous, as this term is used herein, if they have an alignment score of at more than 5 (in standard deviation units) using the program ALIGN with the mutation data matrix and a gap penalty of 6 or greater. The two sequences or parts thereof are more preferably homologous if their amino acids are greater than or equal to 50% identical when optimally aligned using the ALIGN program.

[0047] The term "corresponds to" is used herein to mean that a polynucleotide sequence is homologous (e.g., is identical, not strictly evolutionarily related) to all or a portion of a reference polynucleotide sequence that encodes a polypeptide or its complement, or that a polypeptide sequence is identical in sequence or function to a reference polypeptide sequence. For illustration, the nucleotide sequence "TATAC" corresponds to a reference sequence "TATAC" and is complementary to a reference sequence "GTATA".

[0048] The following terms are used to describe the sequence relationships between two or more polynucleotides: "reference sequence", "comparison window", "sequence identity", "percentage of sequence identity", and "substantial identity". A "reference sequence" is a defined sequence used as a basis for a sequence comparison; a reference sequence may be a subset of a larger sequence, for example, as a segment of a full-length cDNA or gene sequence given in a sequence listing, or may comprise a complete cDNA or gene sequence. Generally, a reference sequence is at least 20 nucleotides in length, frequently at least 25 nucleotides in length, and often at least 50 nucleotides in length. Since two polynucleotides may each (1) comprise a sequence (i.e., a portion of the complete polynucleotide sequence) that is similar between the two polynucleotides, and (2) may further comprise a sequence that is divergent between the two polynucleotides, sequence comparisons between two (or more) polynucleotides are typically performed by comparing sequences of the two polynucleotides over a "comparison window" to identify and compare local regions of sequence similarity.

[0049] A "comparison window", as used herein, refers to a conceptual segment of at least 20 contiguous nucleotides and wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) of 20 percent or less as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Optimal alignment of sequences for aligning a comparison window may be conducted by using local homology algorithms or by a search for similarity method, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA Genetics Software Package or by inspection, and the best alignment (i.e., resulting in the highest percentage of homology over the comparison window) generated by the various methods is selected.

[0050] The term "sequence identity" means that two polynucleotide sequences are identical (i.e., on a nucleotide-by-nucleotide basis) over the window of comparison. The term "percentage of sequence identity" means that two polynucleotide sequences are identical (i.e., on a nucleotide-by-nucleotide basis) over the window of comparison. The term "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (e.g., A, T, C, G, U, or I) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (i.e., the window size), and multiplying the result by 100 to yield the percentage of sequence identity. The terms "substantial identity" as used herein denote a characteristic of a polynucleotide sequence, wherein the polynucleotide comprises a sequence that has at least 85 percent sequence identity, preferably at least 90 to 95 percent sequence identity, more usually at least 99 percent sequence identity as compared to a reference sequence over a comparison window of at least 20 nucleotide positions, frequently over a window of at least 20-50 nucleotides, wherein the percentage of sequence identity is calculated by comparing the reference sequence to the polynucleotide sequence which may include deletions or additions which total 20 percent or less of the reference sequence over the window of comparison.

[0051] As applied to polypeptides, the term "substantial identity" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least about 80% sequence identity, preferably at least about 90% sequence identity, more preferably at least about 95% percent sequence identity, and most preferably at least about 99% sequence identity.

[0052] A "protective immune response" and "prophylactic immune response" are used interchangeably to refer to an immune response which targets an immunogen to which the individual has not yet been exposed or targets a protein associated with a disease in an individual who does not have the disease, such as a tumor associated protein in a patient who does not have a tumor.

[0053] A "therapeutic immune response" refers to an immune response which targets an immunogen to which the individual has been exposed or a protein associated with a disease in an individual who has the disease.

[0054] The term "prophylactically effective amount" is meant to refer to the amount necessary to, in the case of infectious agents, prevent an individual from developing an infection, and in the case of diseases, prevent an individual from developing a disease.

[0055] The term "therapeutically effective amount" is meant to refer to the amount necessary to, in the case of infectious agents, reduce the level of infection in an infected individual in order to reduce symptoms or eliminate the infection, and in the case of diseases, to reduce symptoms or cure the individual.

[0056] "Inducing an immune response against an immunogen" is meant to refer to induction of an immune response in a nave individual and induction of an immune response in an individual previously exposed to an immunogen wherein the immune response against the immunogen is enhanced.

[0057] As used herein, "substantially pure" means an object species is the predominant species present (i.e., on a molar basis it is more abundant than any other individual species in the composition), and preferably a substantially purified fraction is a composition wherein the object species comprises at least about 50 percent (on a molar basis) of all macromolecular species present. Generally, a substantially pure composition will comprise more than about 80 percent of all macromolecular species present in the composition, more preferably more than about 85%, about 90%, about 95%, and about 99%. Most preferably, the object species is purified to essential homogeneity (contaminant species cannot be detected in the composition by conventional detection methods) wherein the composition consists essentially of a single macromolecular species.

[0058] "Transfected," "transformed" or "transgenic" is used herein to include any host cell or cell line, which has been altered or augmented by the presence of at least one recombinant DNA sequence. The host cells of the present invention are typically produced by transfection with a DNA sequence in a plasmid expression vector, as an isolated linear DNA sequence, or infection with a recombinant viral vector.

Exemplary Viruses and Methods of the Invention

[0059] The invention provides isolated vectors, e.g., plasmids, which encode proteins of negative-sense, single stranded RNA viruses and/or express vRNA from recombinant nucleic acid corresponding to sequences for mutant negative-sense, single stranded RNA viruses. When introduced into a cell, a combination of these vectors is capable of yielding recombinant infectious, biologically contained virus. Thus, the invention includes host cells that produce recombinant infectious, biologically contained virus of the invention. In one embodiment, the invention provides isolated vectors, e.g., plasmids, which encode filovirus proteins and/or express mutant filovirus vRNA which, when introduced into a cell, are capable of yielding recombinant infectious, biologically contained filovirus. The invention includes host cells that transiently or stably produce recombinant infectious, biologically contained filovirus, including helper cells, and isolated recombinant filovirus prepared by the methods disclosed herein.

[0060] The vectors of the invention include those for mRNA production and vRNA production. In one embodiment, the vectors include filovirus DNA, for example, vectors for mRNA production with sequences corresponding to one or more open reading frames encoding filovirus proteins, or vectors for vRNA production that include a deletion of the full-length genomic sequence, which deletion includes internal filovirus sequences corresponding to at least a portion of one open reading frame. The RNA produced from the vRNA vector is capable of being packaged into virions in the presence of filovirus proteins but as part of the resulting virion, is not capable of being replicated and so does not result in virus production when that virion is introduced to a cell that otherwise supports filovirus replication and which cell does not express at least one filovirus protein in trans, e.g., a cell that is not a filovirus helper cell.

[0061] For example, *Ebolaviruses* possess a negative-sense, nonsegmented RNA genome, approximately 19 kilobases in length that encodes seven structural proteins and at least one non-structural protein (Sanchez et al., 2007). NP, viral protein (VP)35, VP30, and L, the RNA-dependent RNA polymerase, are components of the nucleocapsid involved in viral replication and transcription (Muhlberger

et al., 1999). VP40 is the matrix protein and is involved in viral budding (Harty et al., 2000; Panchal et al., 2003). VP24 is involved in the formation of nucleocapsids composed of NP, VP35 and viral RNA (Huang et al., 2002). The only viral surface glycoprotein, GP, plays a role in viral attachment and entry (Chan et al., 2001; Manicassamy et al., 2005; Shimojima et al., 2006; Chandran et al., 2005). Candidate sequences for deletion/mutation/insertion and optional replacement with heterologous sequences include but are not limited to *Ebola virus* VP30 sequences or corresponding sequences in other negative-sense, single stranded RNA viruses, e.g., sequences for nonstructural, nonpolymerase and/or nonglycosylated viral proteins or non-coding regions. The vectors may include gene(s) or portions thereof other than those of a negative-sense, single stranded RNA virus such as a filovirus (heterologous sequences), which genes or portions thereof are intended to be expressed in a host cell, either as a protein or incorporated into vRNA. Thus, a vector of the invention may include in addition to viral sequences, for instance, filovirus sequences, a gene or open reading frame of interest, e.g., a heterologous gene for an immunogenic peptide or protein useful as a vaccine or a therapeutic protein.

[0062] If more than one vector is employed, the vectors may be physically linked or each vector may be present on an individual plasmid or other, e.g., linear, nucleic acid delivery vehicle. The vectors or plasmids may be introduced to any host cell, e.g., a eukaryotic cell such as a mammalian cell, that supports viral replication. Host cells useful to prepare virus of the invention include but are not limited to insect, avian or mammalian host cells such as canine, feline, equine, bovine, ovine, or primate cells including simian or human cells. In one embodiment, the host cell is one that is approved for vaccine production.

[0063] The viruses produced by methods described herein are useful in viral mutagenesis studies, drug screening and in the production of vaccines (e.g., for AIDS, influenza, hepatitis B, hepatitis C, rhinovirus, filoviruses, malaria, herpes, and foot and mouth disease) and gene therapy vectors (e.g., for cancer, AIDS, adenosine deaminase, muscular dystrophy, ornithine transcarbamylase deficiency and central nervous system tumors). In particular, infectious, biologically contained filovirus of the invention which induces strong humoral and cellular immunity may be employed as a vaccine vector, as they are unlikely to give rise to infectious recombinant virus.

[0064] Thus, a virus for use in medical therapy (e.g., for a vaccine or gene therapy) is provided. For example, the invention provides a method to immunize an animal against a pathogen, e.g., a bacteria, virus such as *Ebola virus,* or parasite, or a malignant tumor. The method comprises administering to the animal an effective amount of at least one isolated virus of the invention which encodes and expresses, or comprises nucleic acid for an immunogenic peptide or protein of a pathogen or tumor, optionally in combination with an adjuvant, effective to immunize the animal.

[0065] To prepare expression cassettes for transformation herein, the recombinant DNA sequence or segment may be circular or linear, double-stranded or single-stranded. A DNA sequence which encodes an RNA sequence that is substantially complementary to a mRNA sequence encoding a gene product of interest is typically a "sense" DNA sequence cloned into a cassette in the opposite orientation (i.e., 3_ to 5_ rather than 5_ to 3_). Generally, the DNA sequence or segment is in the form of chimeric DNA, such as plasmid DNA, that can also contain coding regions flanked by control sequences which promote the expression of the DNA in a cell. As used herein, "chimeric" means that a vector comprises DNA from at least two different species, or comprises DNA from the same species, which is linked or associated in a manner which does not occur in the "native" or wild-type of the species.

[0066] Aside from DNA sequences that serve as transcription units, or portions thereof, a portion of the DNA may be untranscribed, serving a regulatory or a structural function. For example, the DNA may itself comprise a promoter that is active in eukaryotic cells, e.g., mammalian cells, or in certain cell types, or may utilize a promoter already present in the genome that is the transformation target of the lymphotropic virus. Such promoters include the CMV promoter, as well as the SV40 late promoter and retroviral LTRs (long terminal repeat elements), e.g., the MMTV, RSV, MLV or HIV LTR, although many other promoter elements well known to the art may be employed in the practice of the invention.

[0067] Other elements functional in the host cells, such as introns, enhancers, polyadenylation sequences and the like, may also be a part of the recombinant DNA. Such elements may or may not be necessary for the function of the DNA, but may provide improved expression of the DNA by affecting transcription, stability of the mRNA, or the like. Such elements may be included in the DNA as desired to obtain the optimal performance of the transforming DNA in the cell.

[0068] The recombinant DNA to be introduced into the cells may contain either a selectable marker gene or a reporter gene or both to facilitate identification and selection of transformed cells from the population of cells sought to be transformed. Alternatively, the selectable marker may be carried on a separate piece of DNA and used in a co-transformation procedure. Both selectable markers and reporter genes may be flanked with appropriate regulatory sequences to enable expression in the host cells. Useful selectable markers are well known in the art and include, for example, antibiotic and herbicide-resistance genes. such as neo, hot, dhfr, bar, aroA, puro, hyg, dapA and the like. See also, the genes listed on Table 1 of Lundquist et al. (U.S. Pat. No. 5,848,956).

[0069] Reporter genes are used for identifying potentially transformed cells and for evaluating the functionality of regulatory sequences. Reporter genes which encode for easily assayable proteins are well known in the art. In general, a reporter gene is a gene which is not present in or expressed by the recipient organism or tissue and which encodes a protein whose expression is manifested by some easily detectable property, e.g., enzymatic activity. Exemplary reporter genes include the chloramphenicol acetyl transferase gene (cat) from Tn9 of *E. coli,* the beta-glucuronidase gene (gus) of the uidA locus of *E. coli,* the green, red, or blue fluorescent protein gene, and the luciferase gene. Expression of the reporter gene is assayed at a suitable time after the DNA has been introduced into the recipient cells.

[0070] The general methods for constructing recombinant DNA which can transform target cells are well known to those skilled in the art, and the same compositions and methods of construction may be utilized to produce the

DNA useful herein. For example, Sambrook et al., *Molecular Cloning: A Laboratory Manual* (2002) provides suitable methods of construction.

[0071] The recombinant DNA can be readily introduced into the host cells, e.g., mammalian, yeast or insect cells, by transfection with an expression vector comprising the recombinant DNA by any procedure useful for the introduction into a particular cell, e.g., physical or biological methods, to yield a transformed (transgenic) cell having the recombinant DNA so that the DNA sequence of interest is expressed by the host cell. In one embodiment, at least one of the recombinant DNA which is introduced to a cell is maintained extrachromosomally. In one embodiment, at least one recombinant DNA is stably integrated into the host cell genome.

[0072] Physical methods to introduce a recombinant DNA into a host cell include calcium-mediated methods, lipofection, particle bombardment, microinjection, electroporation, and the like. Biological methods to introduce the DNA of interest into a host cell include the use of DNA and RNA viral vectors. Viral vectors, e.g., retroviral or lentiviral vectors, have become a widely used method for inserting genes into eukaryotic, such as mammalian, e.g., human, cells. Other viral vectors useful to introduce genes into cells can be derived from poxviruses, e.g., vaccinia viruses, herpes viruses, adenoviruses, adeno-associated viruses, baculoviruses, and the like.

[0073] To confirm the presence of the recombinant DNA sequence in the host cell, a variety of assays may be performed. Such assays include, for example, molecular biological assays well known to those of skill in the art, such as Southern and Northern blotting, RT-PCR and PCR; biochemical assays, such as detecting the presence or absence of a particular gene product, e.g., by immunological means (ELISAs and Western blots) or by other molecular assays.

[0074] To detect and quantitate RNA produced from introduced recombinant DNA segments, RT-PCR may be employed. In this application of PCR, it is first necessary to reverse transcribe RNA into DNA, using enzymes such as reverse transcriptase, and then through the use of conventional PCR techniques amplify the DNA. In most instances PCR techniques, while useful, will not demonstrate integrity of the RNA product. Further information about the nature of the RNA product may be obtained by Northern blotting. This technique demonstrates the presence of an RNA species and gives information about the integrity of that RNA. The presence or absence of an RNA species can also be determined using dot or slot blot Northern hybridizations. These techniques are modifications of Northern blotting and only demonstrate the presence or absence of an RNA species.

[0075] While Southern blotting and PCR may be used to detect the recombinant DNA segment in question, they do not provide information as to whether the recombinant DNA segment is being expressed. Expression may be evaluated by specifically identifying the peptide products of the introduced DNA sequences or evaluating the phenotypic changes brought about by the expression of the introduced DNA segment in the host cell.

[0076] The recombinant viruses described herein have modifications in genomic sequences relative to a corresponding wild-type viral genome, i.e., the genome of the recombinant virus has a modification which includes a deletion, and optionally an insertion, in a region corresponding to sequences for a viral protein that is associated with transcription, is nonstructural or nonglycosylated. The mutation in the viral genome is effective to inhibit or prevent production of at least one functional viral protein from that genome when those sequences are present in a nontransgenic cell which supports viral replication. In one embodiment, the deletion includes from 1 up to thousands of nucleotides, e.g., 1%, 10%, 50%, 90% or more of sequences corresponding to the coding region for the viral protein. In one embodiment, the deleted sequences correspond to sequences with a substantial identity, e.g., at least 80% or more, e.g., 85%, 90% or 95% and up to 100% or any integer in between, nucleic acid sequence identity, to VP30 sequences and/or GP/sGP sequences. In one embodiment, the deletion includes from 1 up to hundreds of nucleotides, e.g., 1%, 10%, 50%, 90% or more of sequences corresponding to at least non-coding sequences between NP coding sequences and VP35 coding sequences. In one embodiment, the deleted sequences correspond to sequences with a substantial identity, e.g., at least 80% or more, e.g., 85%, 90% or 95% and up to 100% or any integer in between, nucleic acid sequence identity, to non-coding sequences between NP coding sequences and VP35 coding sequences.

[0077] In one embodiment, the viral genome in an infectious, replication-incompetent negative-sense, single-stranded RNA virus of the invention includes a deletion in sequences corresponding to those in a wild-type viral genome for a protein that is associated with transcription or is nonstructural or nonglycoslyated, and includes heterologous sequences that are nontoxic to host cells including cells in an organism to be immunized. In one embodiment, the heterologous sequence is a marker sequence, a selectable sequence or other sequence which is detectable or capable of detection, e.g., GFP or luciferase, or a selectable gene such as an antibiotic resistance gene, e.g., a hygromycin B resistance gene or neomycin phosphotransferase gene, which marker gene or selectable gene is not present in the host cell prior to introduction of the vector.

Pharmaceutical Compositions

[0078] Pharmaceutical compositions of the present invention, suitable for inoculation, e.g., nasal, parenteral or oral administration, such as by intravenous, intramuscular, intranasal, topical or subcutaneous routes, comprise one or more virus isolates, e.g., one or more recombinant infectious, biologically contained negative-sense, single stranded RNA virus isolates, optionally further comprising sterile aqueous or non-aqueous solutions, suspensions, and emulsions. The compositions can further comprise auxiliary agents or excipients, as known in the art. The composition is generally presented in the form of individual doses (unit doses). Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and/or emulsions, which may contain auxiliary agents or excipients known in the art. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Carriers or occlusive dressings can be used to increase skin permeability and enhance antigen absorption. Liquid dosage forms for oral administration may generally comprise a liposome solution containing the liquid dosage form. Suitable forms for suspending liposomes include emulsions, suspensions, solutions, syrups, and elixirs containing inert diluents commonly used in the art, such as purified water.

Besides the inert diluents, such compositions can also include adjuvants, wetting agents, emulsifying and suspending agents, or sweetening, flavoring, or perfuming agents.

[0079] When a composition is used for administration to an individual, it can further comprise salts, buffers, adjuvants, or other substances which are desirable for improving the efficacy of the composition. p For vaccines, adjuvants, substances which can augment a specific immune response, can be used. Normally, the adjuvant and the composition are mixed prior to presentation to the immune system, or presented separately, but into the same site of the organism being immunized.

[0080] In one embodiment, the pharmaceutical composition is part of a controlled release system, e.g., one having a pump, or formed of polymeric materials (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, N.Y. (1984); Ranger & Peppas, *J. Macromol. Sci. Rev. Macromol. Chem.* 22:61 (1983); see also Levy et al., *Science* 228:190 (1985); During et al., *Ann. Neurol.,* 25:351 (1989); Howard et al., *J. Neurosurg.,* 71:105 (1989)). Other controlled release systems are discussed in the review by Langer (*Science,* 2.42:1527 (1990)).

[0081] The pharmaceutical compositions comprise a therapeutically effective amount of the 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 pharmacopeiae 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. Saline solutions and aqueous dextrose and glycerol solutions can also be 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. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. These compositions can be formulated as a suppository. Oral formulation can include 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 "Remington's Pharmaceutical Sciences" by E. W. Martin. Such compositions will contain a therapeutically effective amount of the virus, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

[0082] The compositions may be systemically administered, e.g., orally or intramuscularly, in combination with a pharmaceutically acceptable vehicle such as an inert diluent. For oral administration, the virus may be combined with one or more excipients and used in the form of ingestible capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions should contain at least 0.1% of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 2 to about 60% of the weight of a given unit dosage form. The amount of active compound in such useful compositions is such that an effective dosage level will be obtained.

[0083] The compositions may also contain the following: binders such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, fructose, lactose or aspartame or a flavoring agent such as peppermint, oil of wintergreen, or cherry flavoring may be added. Various other materials may be present. For instance, a syrup or elixir may contain the virus, sucrose or fructose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavoring such as cherry or orange flavor. Of course, any material used in preparing any unit dosage form, including sustained-release preparations or devices, should be pharmaceutically acceptable and substantially non-toxic in the amounts employed.

[0084] The composition also be administered intravenously or intraperitoneally by infusion or injection. Solutions of the virus can be prepared in water or a suitable buffer, optionally mixed with a nontoxic surfactant. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, triacetin, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of undesirable microorganisms.

[0085] The pharmaceutical dosage forms suitable for injection or infusion can include sterile aqueous solutions or dispersions or sterile powders comprising the active ingredient which are adapted for the extemporaneous preparation of sterile injectable or infusible solutions or dispersions, optionally encapsulated in liposomes. In all cases, the ultimate dosage form should be sterile, fluid and stable under the conditions of manufacture and storage. The liquid carrier or vehicle can be a solvent or liquid dispersion medium comprising, for example, water, ethanol, a polyol (for example, glycerol, propylene glycol, liquid polyethylene glycols, and the like), vegetable oils, nontoxic glyceryl esters, and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the formation of liposomes, by the maintenance of the required particle size in the case of dispersions or by the use of surfactants. The prevention of the action of undesirable microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, buffers or sodium chloride.

[0086] Sterile injectable solutions are prepared by incorporating the virus in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filter sterilization.

[0087] Useful liquid carriers include water, alcohols or glycols or water-alcohol/glycol blends, in which the present viruses can be dissolved or dispersed at effective levels, optionally with the aid of non-toxic surfactants. Adjuvants such as fragrances and additional antimicrobial agents can be added to optimize the properties for a given use. The resultant liquid compositions can be applied from absorbent pads, used to impregnate bandages and other dressings, or sprayed onto the affected area using pump-type or aerosol sprayers.

[0088] Useful dosages of the viruses of the invention can be determined by comparing their in vitro activity and in vivo activity in animal models.

Pharmaceutical Purposes

[0089] The administration of the composition may be for either a "prophylactic" or "therapeutic" purpose. When provided prophylactically, the compositions of the invention which are vaccines are provided before any symptom or clinical sign of a pathogen infection becomes manifest. The prophylactic administration of the composition serves to prevent or attenuate any subsequent infection. When provided prophylactically, the gene therapy compositions of the invention, are provided before any symptom or clinical sign of a disease becomes manifest. The prophylactic administration of the composition serves to prevent or attenuate one or more symptoms or clinical signs associated with the disease.

[0090] When provided therapeutically, a viral vaccine is provided upon the detection of a symptom or clinical sign of actual infection. The therapeutic administration of the compound(s) serves to attenuate any actual infection. When provided therapeutically, a gene therapy composition is provided upon the detection of a symptom or clinical sign of the disease. The therapeutic administration of the compound (s) serves to attenuate a symptom or clinical sign of that disease.

[0091] Thus, a vaccine composition of the present invention may be provided either before the onset of infection (so as to prevent or attenuate an anticipated infection) or after the initiation of an actual infection. Similarly, for gene therapy, the composition may be provided before any symptom or clinical sign of a disorder or disease is manifested or after one or more symptoms are detected.

[0092] A composition is said to be "pharmacologically acceptable" if its administration can be tolerated by a recipient mammal. Such an agent is said to be administered in a "therapeutically effective amount" if the amount administered is physiologically significant. A composition of the present invention is physiologically significant if its presence results in a detectable change in the physiology of a recipient patient, e.g., enhances at least one primary or secondary humoral or cellular immune response against at least one strain of a virus.

[0093] The "protection" provided need not be absolute, i.e., the influenza infection need not be totally prevented or eradicated, if there is a statistically significant improvement compared with a control population or set of mammals. Protection may be limited to mitigating the severity or rapidity of onset of symptoms or clinical signs of the virus infection.

Pharmaceutical Administration

[0094] A composition of the present invention may confer resistance to one or more pathogens, e.g., one or more virus, bacterium or parasite strains, by either passive immunization or active immunization. In active immunization, a live vaccine composition is administered prophylactically to a host (e.g., a mammal), and the host's immune response to the administration protects against infection and/or disease. For passive immunization, the elicited antisera can be recovered and administered to a recipient suspected of having an infection caused by at least one virus strain.

[0095] The present invention thus includes methods for preventing or attenuating a disorder or disease, e.g., an infection by at least one strain of pathogen. As used herein, a vaccine is said to prevent or attenuate a disease if its administration results either in the total or partial attenuation (i.e., suppression) of a clinical sign or condition of the disease, or in the total or partial immunity of the individual to the disease.

[0096] At least one virus isolate of the present invention, may be administered by any means that achieve the intended purposes. For example, administration of such a composition may be by various parenteral routes such as subcutaneous, intravenous, intradermal, intramuscular, intraperitoneal, intranasal, oral or transdermal routes. Parenteral administration can be accomplished by bolus injection or by gradual perfusion over time.

[0097] A typical regimen for preventing, suppressing, or treating a viral related pathology, comprises administration of an effective amount of a vaccine composition as described herein, administered as a single treatment, or repeated as enhancing or booster dosages, for instance, over a period up to and including between one week and about 24 months, or any range or value therein.

[0098] According to the present invention, an "effective amount" of a composition is one that is sufficient to achieve a desired effect. It is understood that the effective dosage may be dependent upon the species, age, sex, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect wanted. The ranges of effective doses provided below are not intended to limit the invention and represent dose ranges.

[0099] Exemplary doses include but are not limited to from about 10^4 to 10^8 FFU or PFU, 10^6 to 10^8 FFU or PFU, 10^6 to 10^{10} FFU or PFU, or 10^8 to 10^{12} FFU or PFU, or more, or from about 10^6 to 10^8 particles, 10^8 to 10^{10} particles, or 10^{10} to 10^{12} particles. In one embodiment a dose is from about 10^4 to 10^8 FFU or PFU, 10^6 to 10^8 FFU or PFU, 10^6 to 10^{10} FFU or PFU, or 10^8 to 10^{10} FFU or PFU.

Exemplary Adjuvants

[0100] Adjuvants include but are not limited to aluminum, water in oil (W/O) emulsions, oil in water (O/W) emulsions, ISCOM, liposomes, nano- or micro-particles, muramyl di- and/or tripeptides, saponin, non-ionic block co-polymers, lipid A, cytokines, bacterial toxins, carbohydrates, and derivatized polysaccharides and a combination of two or more these adjuvants in an Adjuvant System (AS).

[0101] Exemplary classes of adjuvants include but are not limited to agonists of TLR3, e.g., poly (I:C), agonists of TLR4, e.g., one or more components of bacterial lipopolysaccharide, e.g., monophosphoryl lipid A (MPLA), MPL®, and synthetic derivatives, e.g., E6020,agonists of TLR5, e.g., bacterial flagellin), agonists of TLR7, 8, e.g., single stranded RNA or imidazoquinolines (e.g., imiquimod, gardiquimod and R848),agonists of TLR9, e.g., CpG oligonucleotides and ISS immunostimulatory sequences, as well as imidazoquinolines, agonists of the NLRP3 inflammasome, e.g., chitosan, and dual TLR1/2 agonists, e.g., Pam3CSK4, a lipopeptide.

[0102] In one embodiment, the adjuvant comprises saponin, a natural product derived from tree bark, which may be combined with cholesterol or a cholesterol like molecule, e.g., squalene.

[0103] In one embodiment, the adjuvant comprises an oil-in-water (O/W) emulsion comprising, for example, MF59 or AS03 and optionally 2% squalene. In one embodiment, the adjuvant comprises two different adjuvants, e.g., MPL and a saponin such as QS21, for example, in liposome.

[0104] In one embodiment, the adjuvant comprises Freund's Incomplete Adjuvant (IFA), MF59®, GLA-SE, IC31®, CAF01 AS03, AS04, or ISA51, and may include α-tocopherol, squalene and/or polysorbate 80 in an oil-in-water emulsion.

[0105] In one embodiment, the adjuvant comprises extracts and formulations prepared from Ayurvedic medicinal plants including but not limited to *Withania somnifera, Emblica officinalis, Panax notoginseng, Tinospora cordifolia* or *Asparagus racemosus.*

[0106] In one embodiment, the adjuvant comprises aluminum salts, saponin, muramyl di- and/or tripeptides, Bordetella pertussis, and/or cytokines.

[0107] In one embodiment, the adjuvant is not alum or an aluminum salt.

[0108] In one embodiment, the adjuvant is mixed with the recombinant filovirus just prior to administration.

Exemplary Antigens

[0109] Exemplary viral glycoproteins include but are not limited to those from *ebolaviruses,* e.g., Zaire, Sudan, Bundibugyo, Tai Forest (formerly known as Côte d'Ivoire), or Reston, *marburgviruses,* arenaviruses such as Lassa virus; or bunyaviruses such a Crimean-Congo Hemorrhagic Fever virus or hantaviruses; or flaviruses such as Dengue virus, Zika virus, or Yellow Fever virus.

[0110] Exemplary parasite antigens include but are not limited to those from *Plasmodium, Leishmania Giardia, Cryptosporidium* or *Cyclospora;*

[0111] Exemplary bacterial antigens include but are not limited to those from Vibrio, e.g. *V. cholera* or *Mycobacterium.*

Exemplary Methods to Prepare the Recombinant Filoviruses

Methods and Materials

[0112] Cells and cell lines. Vero cells (green monkey kidney cells) are grown in Eagle's minimal essential medium (MEM) supplemented with 10% fetal calf serum (FCS), L.-glutamine, vitamins, nonessential amino acid solution and antibiotics. The VeroVP30 cell line is established by cotransfecting Vero cells with pCAG-VP30 (for the expression of VP30) and pPur, a protein expression plasmid for the puromycin resistance gene (Clontech, Mountain View, Calif.), using the transfection reagent TransIT LT-1 (Mirus, Madison, Wis.). Two days after transfection, puromycin-resistant cells are selected with 5 μg/mL puromycin (Sigma, St. Louis, Mo.). Individual cell clones are screened for VP30 expression by flow cytometry with a polyclonal peptide antibody to VP30.

[0113] Human embryonic kidney 293T cells are grown in high-glucose Dulbecco's modified Eagle medium containing 10% FCS, L-glutamine, and antibiotics. All cells are maintained at 37° C. and 5% CO_2.

[0114] Flow cytometry. Cells are detached in phosphate-buffered saline (PBS) containing 0.02% EDTA and then washed once with cold PBS supplemented with 2% FCS and 0.1% sodium azide (wash buffer). Cells are incubated with a VP30 antibody on ice for 20 minutes. After washing in buffer, the cells are further incubated with a secondary antibody labeled with fluorescent isothiocyanate (Zymed Laboratories, Carlsbad, Calif.). They are then washed with buffer and analyzed by FACSCalibur with Cell Quest software (Becton Dickinson, Franklin Lakes, N.J.).

[0115] Generation of *Ebola*ΔVP30 viruses. The plasmid pTM-T7G-Ebo-Rib, containing the full-length *Ebolavirus* cDNA flanked by T7 RNA polymerase promoter and ribozyme sequences, is described in Newmann et al. (2002). First, a fragment encompassing nucleotides 6180 to 10942 (numbers refers to the positive-sense antigenome) is subcloned into a kanamycin-resistant cloning vector. Next, the VP30 ORF is replaced with those encoding neo or eGFP, respectively, by a series of overlapping PCR amplification steps using Pfu Turbo (Stratagene, La Jolla, Calif.). The altered subgenomic fragments are transferred back into the full-length *Ebolavirus* cDNA plasmid using two unique restriction sites, SalI and SacI (FIG. **1**). The resultant plasmids, designated pTM-*Ebola*ΔVP30-neo or -eGFP, are sequenced to verify the replacement of the VP30 ORF and the lack of any unwanted mutations.

[0116] To artificially generate *Ebolavirus,* 5×10^5 293T cells are transfected with 1.0 μg pTM-*Ebola*ΔVP30, 2.0 μg pCAG-L, 1.0 μg pCAG-NP, 0.5 μg pCAG-VP35, 0.5 μg pCAG-VP30, and 1.0 μg pCAG-T7 pol, using TransIT LT1 (Mirus, Madison, Wis.) in BSL-4 containment (Neumann et al., 2002). Five days after transfection, the supernatant is harvested, cellular debris removed by low speed centrifugation, and the virus amplified in VeroVP30 cells at 37° C. and 5% CO_2 with propagation medium containing 2% FCS in MEM supplemented with L-glutamine, vitamins, nonessential amino acid solution and antibiotics without puromycin.

[0117] Plaque assay and immunostaining assay. To determine the titers of wild-type *Ebolavirus* or *Ebola*ΔVP30 viruses, tenfold dilutions of the viruses are absorbed to confluent VeroVP30 or wild-type Vero cells for 1 hour at 37° C., after which any unbound virus was removed by washing cells with propagation medium. The cells are then overlaid with propagation medium containing 1.5% methyl cellulose (Sigma). Seven days after infection, cells are fixed with 10% buffered formaldehyde, taken out of BSL-4, permeabilized with 0.25% Triton X-100 in PBS for 10 minutes, and blocked with 4% goat serum and 1% bovine serum albumin (BSA) in PBS for 60 minutes. Cells are then incubated for 60 minutes with a 1:1000 dilution of a mouse anti-VP40 monoclonal antibody, washed with PBS, and incubated for 60 minutes with a 1:1000 dilution of an antimouse IgG-peroxidase-conjugated secondary antibody (Kirkegaard & Perry Laboratories Inc., Gaithersburg, Md.). After washing with PBS, cells are incubated with 3,3'-diaminobenzidine tetrahydrochloride (DAB, Sigma) in PBS. The reaction is stopped by rinsing cells with water.

[0118] Western blotting. Partially purified virus is resuspended in lysis buffer (50 mM Tris-HCl [pH 7.5], 150 mM NaCl, 0.5% Triton X-100, and 0.1% SDS) containing protease inhibitors (complete protease inhibitor cocktails [Roche]) was incubated at 100° C. for 5 minutes, taken out of BSL-4, and separated on 4-20% polyacrylamide gels. Resolved proteins are transferred to Western polyvinylidine difluoride membranes (Schleicher & Schuell, Sanford, Me.) and blocked overnight at 4° C. with 5% skim milk in PBST (0.05% Tween 20 [Sigma] in PBS). Blots are incubated with

primary antibodies (a mouse anti-NP antibody, a rabbit anti-VP35 antibody, a rabbit anti-VP40 antibody, a mouse anti-GP antibody, a rabbit anti-VP30 antibody, or a mouse anti-VP24 antibody) for 60 minutes at room temperature, washed three times with PBST, incubated with the appropriate secondary antibody conjugated to horseradish peroxidase (Zymed) for 60 minutes, and finally washed three times with PBST. Blots were then incubated in Lumi-Light Western blotting substrate (Roche, Indianapolis. Ind.) and exposed to X-ray film (Kodak, Rochester, N.Y.).

[0119] RNA isolation and RT-PCR. Cell culture supernatant from virus-infected VeroVP30 cells is inactivated with guanidinium isothiocyanate buffer and taken out of BSL-4. Viral RNA is isolated with the RNeasy Mini kit (Qiagen, Valencia, Calif.). RT-PCR is carried out with the RobusT One-Step RT-PCR kit (Finnzyme, Espoo, Finland), using 1 μg of isolated RNA and *Ebolavirus*-specific primers. The resultant PCR products are cloned into pT7Blue (Novagen, San Diego, Calif.) and sequenced.

[0120] Transmission electron microscopy. Ultrathin-section electronmicroscopy is performed as described in Node et al. (2002). Briefly, at 36 hours postinfection, VeroVP30 cells infected with *Ebola*ΔVP30-neo virus are fixed and inactivated with 2.5% glutaraldehyde in 0.1 M cacodylate buffer, taken out of BSL-4 and postfixed with 2% osmium tetroxide in the same buffer. Cells are then dehydrated with a series of ethanol gradients followed by propylene oxide, before being embedded in Epon 812 Resin mixture (TAAB Laboratories Equipment Ltd., Berkshire, UK). Thin sections are stained with 2% uranyl acetate and Raynold's lead, and examined under a HITACHI H-7500 electron microscope at 80 kV.

[0121] Selection of escape mutants. *Ebola*ΔVP30-eGFP is diluted tenfold (10^{-1} to 10^{-6}) and incubated with the indicated mAbs at a concentration of 250 to 500 μg of mAb/mL at 37° C. for 60 minutes. The virus/mAb mixtures are inoculated onto VeroVP30 cells for 60 minutes. Viruses are amplified for 5 days in the presence of antibodies. Then, viruses that grow in the presence of mAbs (as determined by GFP expression) are harvested at the highest virus-positive dilution and passaged for a total of 3-6 times in the presence of antibodies. Viral RNA is isolated, RT-PCR amplified, and the GP sequence determined by sequence analysis.

[0122] Generation and passage of *Ebola*ΔVP30-neo virus. Previously a full-length cDNA clone of the Zaire *Ebolavirus*-Mayinga was generated (Newmann et al., 2002). Using a subgenomic fragment that encompasses nucleotides 6180 to 10942 of the viral genome (numbers refers to the positive-sense antigenome), the ORF for VP30 was replaced with that of neomycin (neo), using a series of overlapping PCR amplification steps. After confirmation of the authenticity of the PCR fragments by sequence analysis, the altered subgenomic fragment was inserted into the full-length *Ebolavirus* cDNA construct via unique SalI and SacI restriction sites (FIG. **1**), resulting in an *Ebolavirus* cDNA genome deficient in the VP30 ORF. The artificial generation of *Ebolavirus* from plasmids is afforded by flanking this viral cDNA with T7 RNA polymerase promoter and hepatitis delta virus ribozyme sequences (Neumann et al., 2002).

[0123] To amplify VP30-deficient *Ebolaviruses,* a stable Vero E6 cell line (designated VeroVP30) was established by cotransfecting Vero cells with two protein expression plasmids encoding VP30 (pCAG-VP30) and puromycin (pPur, Clontech), and selecting cell clones resistant to 5.0 μg/mL of puromycin. VP30 expression in individual clones was determined by flow cytometry with antibodies to VP30. The clone with the highest percentage of VP30-expressing cells (>90% as measured by flow cytometry) was used in further studies to amplify *Ebola*ΔVP30 viruses.

[0124] Briefly, human embryonic kidney (293T) cells were transfected with a plasmid for the transcription of the VP30-deficient *Ebolavirus* RNA, with plasmids for the expression of the *Ebolavirus* NP, VP30, VP35, and L proteins, and with a plasmid for the expression of T7 RNA polymerase. Five days after transfection, VeroVP30 cells were incubated with undiluted supernatant derived from plasmid-transfected cells. Seven days later, the supernatant was harvested, diluted tenfold, and used to infect fresh VeroVP30 cells for the next passage. A total of seven passages were carried out, using the highest dilution of the inoculum that still produced replicating viruses for each passage. The presence of replicating virus was assessed by cytopathic effects (CPE) and immunostaining of infected VeroVP30 cells with an antibody to VP40. As a control, we also incubated the supernatants from each passage with wild-type Vero cells. As expected, CPE and viral antigens were undetectable in wild-type Vero cells, demonstrating that replicating *Ebola*ΔVP30-neo virus was confined to VeroVP30 cells.

[0125] Although the manifestation of a CPE in infected VeroVP30 cells suggested the formation of infectious (but biologically contained) *Ebolaviruses,* further evidence was sought for the presence of virions in cell culture supernatant derived from infected VeroVP30 cells. Briefly, 5 days after VeroVP30 cells were infected with *Ebola*ΔVP30-neo virus, supernatant was collected and partially purified over 20% sucrose. The pellet was suspended in PBS and separated on a 4-20% polyacrylamide gel. Western blot analyses were carried out with antibodies specific to the respective *Ebolavirus* protein. All viral proteins (with the exception of L, for which no antibody was available) were detected. Note that VP30 protein in virions originates from VeroVP30 cells while the remaining proteins are encoded by *Ebola*ΔVP30-neo virus. By contrast, no viral proteins were detected in a control sample derived from wild-type Vero cells infected with *Ebola*ΔVP30-neo virus.

[0126] Genetic stability of *Ebola*ΔVP30-neo virus. A major concern with the use of VP30-deficient *Ebolaviruses* is the potential recombination with VP30 sequences integrated into the genome of the VeroVP30 helper cell line. Thus, to assess the genomic stability of *Ebola*ΔVP30-neo virus, three independent passage experiments were performed (seven passages each). While *Ebola*ΔVP30-neo virus replicated in VeroVP30 cells, viral replication was not observed in wild-type Vero cells. Total viral RNA was isolated from the cell culture supernatant of infected VeroVP30 cells after the seventh passage. A viral genomic fragment spanning the neo gene was amplified by RT-PCR, cloned and sequenced. A total of 20 clones were sequenced, and the sequences were identical to that of the *Ebola*ΔVP30 cDNA construct used for virus generation. Hence, there was no evidence of recombination in any of three independent passage experiments, attesting to the genomic stability of the *Ebola*ΔVP30-neo viral genome.

[0127] To further demonstrate the biosafety of *Ebola*ΔVP30-neo virus, *Ebola*ΔVP30-neo virus was collected after seven consecutive passages in VeroVP30 cells and this virus used for three consecutive "blind" passages in

wild-type Vero cells. Briefly, Vero cells were infected at a multiplicity of infection (m.o.i.) of 5 with Ebola4VP30-neo virus (passage 7). Six days later, supernatant was used for the next "blind" passage as well as for Western blot analysis. No viral NP protein was detected after any of the "blind" passages (data not shown). After three consecutive "blind" passages, plaque assays and immunostaining were carried out in wild-type Vero cells to confirm the absence of replicating *Ebolavirus*. As expected, replicating virus was not detected. Collectively, these data further attest to the biosafety of the *Ebola*ΔVP30 system.

[0128] Growth kinetics of *Ebola*ΔVP30-neo virus. One of the major concerns raised by providing viral proteins in trans is that their amounts, expression kinetics or both may not match those found in cells infected with wild-type virus, leading to reduced virus titers and/or aberrant virion morphology. To address this potential pitfall, the growth kinetics of *Ebola*ΔVP30-neo virus were compared with that of wild-type *Ebolavirus*. VeroVP30 cells or wild-type Vero cells were infected at a high m.o.i. of 1.0 or a low m.o.i. of 0.01 and supernatant was harvested every 24 hours. Virus titers of *Ebola*ΔVP30-neo were determined in VeroVP30 cells, while virus titers of wild-type *Ebolavirus* were determined in wild-type Vero cells. To determine virus titers, cells were overlaid with 1.5% methylcellulose and 7 days later, assayed for VP40 expression using an immunostaining assay, *Ebola*ΔVP30-neo virus replicated efficiently in VeroVP30 cells at both conditions tested, reaching 10^7 focal-forming units (FFU)/ml on day 6 postinfection. No replication of *Ebola*ΔVP30-neo was detected in wild-type Vero cells; the low titers that were detected for up to three days postinfection likely reflect input virus. Together, these findings attest to the biological confinement of the *Ebola*ΔVP30 system. The replication kinetics of *Ebola*ΔVP30-neo in VeroVP30 cells are similar to those of wild-type *Ebolavirus* in either VeroVP30 (FIG. **3**, top panels, open circles) or wild-type Vero cells (FIG. **3**, bottom panels, open circles), establishing the described approach as a highly efficient method for generating biologically contained *Ebolaviruses.*

[0129] Morphology of *Ebola*ΔVP30-neo virus. Next, the morphology of *Ebola*ΔVP30-neo virus was assessed by transmission electron microscopy (TEM). VeroVP30 cells were infected with *Ebola*ΔVP30-neo virus and fixed 36 hours later. Samples were processed for TEM as described in Node et al. (2002). As shown in FIG. **4** (right panels), the particles budding from VeroVP30 cells infected with *Ebola*ΔVP30-neo virus were indistinguishable in their size and shape from wild-type *Ebolaviruses.* Thus, providing VP30 protein in trans does not have a discernable effect on virion morphology, suggesting that the described system would be suitable for studies of virion formation and budding, for example.

[0130] Taken together, the above results demonstrate that the *Ebola*ΔVP30-neo virus is biologically contained, replicates to high titers in a helper cell line, is genetically stable, and is morphologically indistinguishable from wild-type virions. Having provided proof-of-concept for the generation of biologically contained *Ebolaviruses,* the utility of this strategy in basic research and drug screening applications was assessed.

[0131] Generation of an *Ebola*ΔVP30-eGFP virus and its usefulness for basic research applications. An *Ebola*ΔVP30 virus encoding enhanced green fluorescence protein (eGFP) instead of VP30 was generated (FIG. **1**; designated *Ebola*ΔVP30-eGFP), using the same procedures described above for *Ebola*ΔVP30-neo virus. Analogous to *Ebola*ΔVP30-neo virus, the eGFP variant replicated efficiently with virus titers reaching 8.0×10^7 FFU/mL. Expression of eGFP was observed as early as 10 hours postinfection (data not shown).

[0132] Takada et al. (2003) used replication-competent vesicular stomatitis virus (VSV) pseudotyped with *Ebolavirus* GP and two neutralizing monoclonal antibodies (mAb), 133/3.16 and 226/8.1, to map *Ebolavirus* GP epitopes and to generate escape mutants. To confirm with authentic *Ebolavirus* virions the findings of Takada et al. (2003) based on a VSV-pseudotyping system, escape mutants were generated by amplifying *Ebola*ΔVP30-eGFP virus in the presence of mAb 133/3.16 or 226/8.1. Each of eight escape mutants to mAb 133/3.16 possessed a histidine-to-arginine substitution at position 549 (H549R) in OP, reported by Takada et al. (2003). Using mAb 226/8.1, 12 escape mutants were isolated that all contained an arginine-to-tryptophan substitution at position 134 (R134W), a mutation identical to one identified by Takada et al. (2003). However, the remaining two escape mutations described by Takada et al. (2003) were not detected. Whether this discrepancy in escape mutants reflects differences between the biological systems used or random mutations is presently unclear. Nonetheless, these experiments illustrated one of the ways that biologically contained *Ebolaviruses* could be used in basic research applications.

[0133] Biologically contained *Ebola viruses* lacking the VP30 gene afford a safe, alternative way to study authentic *Ebolavirus,* to develop *Ebolavirus* vaccines, and to screen chemical libraries for compounds that interfere with the *Ebolavirus* life cycle. Indeed, each of the three different biologically contained viruses generated (encoding neomycin or eGFP instead of VP30) was biologically contained, as demonstrated by their ability to replicate in VeroVP30 (a Vero cell line that stably expresses VP30 in trans), but not in wild-type Vero cells. Moreover, virus titers were in the range of 10^7 FFU/mL and hence comparable to those obtained for wild-type *Ebolavirus* (FIG. **3**; Volchov et al., 2001; Neumann et al., 2002; Ebihara et al., 2006) while morphological, biochemical, and virological analyses indicated that the tested properties of *Ebola*ΔVP30 viruses were indistinguishable from those of wild-type *Ebolavirus.*

Exemplary Efficacy Protocols

[0134] *Ebola viruses* (family Filoviridae), cause severe hemorrhagic fever in humans and nonhuman primates with mortality rates up to 90% (Johnson et al., 1977). Currently, there are no licensed vaccines or antivirals available against *Ebola virus.* A vaccine against *Ebola virus* is not only desirable for local populations in the epidemic areas of Africa, but also for health care workers during an outbreak and for post-exposure treatment of laboratory workers after accidental exposure to the virus. A few vaccine candidates have been shown to protect mice, guinea pigs, or nonhuman primates against a lethal challenge of *Ebola virus;* however, each of these candidates has disadvantages, such as lack of protection in nonhuman primates, preexisting immunity against the vector in humans, or potential central nervous system involvement (Reed et al., 2007). Moreover, the current vaccine candidates are based on virus-like particles (VLPs) or virus-vectored vaccines, none of which express

the full components of the viral antigens. On the other hand, the use of live attenuated vaccines may not be feasible for *Ebola virus* from a biosafety perspective. To overcome these potential limitations, biologically contained viruses offer an attractive option since they are biologically safe but provide all the viral antigens.

Materials and Methods

[0135] Cells. VeroVP30 cells are established as described in Example 1 and grown in Eagle's minimal essential medium (MEM) supplemented with 10% fetal calf serum (FCS), L-glutamine, vitamins, non-essential amino acid solution, and 5 μg/mL puromycin (Sigma, St. Louis, Mo.).

[0136] Viruses. The *Ebola*ΔVP30 virus is generated as described in Example 1. Briefly, using the plasmid containing the full-length *Ebola* cDNA genome of the Zaire Mayinga strain of *Ebola virus* (Neumann et al., 2002), the open reading frame (ORF) of VP30 is replaced with the ORF of the drug-resistant gene neomycin. Using *Ebola virus* reverse genetics (Neumann et al., 2002), the *Ebola*ΔVP30 virus is generated and passaged in a Vero cell line stably expressing VP30. *Ebola*ΔVP30 was propagated in VeroVP30 cells in MEM medium as described above, but supplemented with 2% FCS. The virus is harvested six days after infection of the cells at a multiplicity of infection (MOI) of 1 and directly stored at −80° C. Harvested virus is also partially purified by ultracentrifugation at 27,000 rpm for 2 hours over 20% sucrose. The viral pellet was resuspended in sterile PBS and stored at −80° C. Viral titers are determined by plaque assay in confluent VeroVP30 cells overlaid with 2% FCS-MEM containing 1.5% methyl cellulose (Sigma).

[0137] Since wild-type *Ebola virus* does not kill mice, challenge studies are carried out with a mouse-adapted *Ebola virus* (Bray et al., 1998). This virus is generated as described in Ebihara et al., 2006.

[0138] Antibody titers. The levels of *Ebola* glycoprotein (GP)-specific immunoglobulin G (IgG) antibodies in vaccinated mice are examined by using an enzyme-linked immunosorbent assay (ELISA). Briefly, wells of Immulon 2HB plates (Thermon Labsystems, Franklin, Mass.) are coated with purified *Ebola* GP (Takada et al., 2001) and blocked with PBS containing 10 mg/mL bovine serum albumin. After incubation of *Ebola* GP-coated wells with mouse serum from control and vaccinated mice, bound antibodies are detected with goat anti-mouse IgG conjugated to horseradish peroxidase (Kirkegaard & Perry Laboratories Inc., Gaithersburg, Md.) by an ELISA plate reader at an absorbance of 405 nm.

[0139] Intracellular staining and flow cytometry. The number of cytokine-producing $CD8^+$ T cells is determined by intracellular staining as described Murali-Krishna et al. (1998). Briefly, splenocytes are stimulated with the *Ebola* peptide NP_{279}-$_{288}$ (SFKAALSSLA, derived from the nucleoprotein NP; SEQ ID NO:16) (Olinger et al., 2006; Simmons et al., 2004), $VP40_{171-180}$ (YFTFDLTALK, derived from the matrix protein VP40; SEQ ID NO:17), or $GP_{161-169}$ (LYDRLASTV, derived from GP) (Olinger et al., 2005; Warfield et al., 2005) for 5 hours in the presence of brefeldin A and IL-2. Following activation, cells are stained for cell surface $CD8^+$ and intracellular IFNγ by using the Cytofix/Cytoperm kit from BD Biosciences (San Jose, Calif.). The number of cytokine-producing $CD8^+$ T cells is determined by using a FACSCalibur flow cytometer (BD Biosciences).

[0140] Vaccination and challenge. Four-week-old female BALB/c mice (The Jackson Laboratory, Bar Harbor, Me.) are anesthetized with isoflurane and intraperitoneally (IP) inoculated twice at three-week intervals with 10^6 focus forming units (FFU) of sucrose-purified *Ebola*ΔVP30 virus; control mice were simultaneously inoculated with PBS. A second group of mice receives three immunizations (at three-week intervals) with 10^7 FFU of virus harvested from cell culture supernatant, or, as a control, 2% FCS-MEM. Vaccinations are conducted at the University of Wisconsin-Madison. Mice are then transported to the BSL-4 laboratory at the National Microbiology Laboratory of the Public Health Agency of Canada. where they were challenged with 1000 mouse lethal doses 50 (MLD_{50}; i.e., the dose required to kill 50% of infected animals) of mouse-adapted *Ebola virus*. Four days after challenge, viral titers are determined in the serum of three control and three vaccinated mice from each group. The remaining mice were monitored for survival for 28 days.

[0141] Antibody response of mice immunized with *Ebola*ΔVP30 virus. To assess the *Ebola*ΔVP30 virus as a potential vaccine, its immunogenicity in mice was determined. Mice vaccinated with the *Ebola*ΔVP30 virus did not show any signs of disease, demonstrating the lack of pathogenicity of the *Ebola*ΔVP30 virus. When serum samples, collected two weeks after each vaccination to determine the levels of antibodies to the *Ebola* glycoprotein (GP), were tested for IgG antibody by ELISA with purified GP, vaccinated animals showed elevated levels of antibody titers against GP after the first vaccination compared to control mice; these antibody titers further increased after the second and third vaccinations. This finding demonstrates the ability of the biologically contained *Ebola*ΔVP30 virus to elicit antibodies to GP.

[0142] $CD8^+$ T-cell responses in vaccinated mice. The cellular response to vaccination in mice was examined. Mice were vaccinated as described above. Eight days after the second immunization, four vaccinated and two control mice were euthanized and their spleens removed. Splenocytes were isolated and stimulated with the *Ebola* peptide $NP_{279-288}$ (SFKAALSSLA), $VP40_{171-180}$ (YFTFDLTALK) or $GP_{161-169}$ (LYDRLASTV) for 5 hours in the presence of brefeldin A and IL-2. Vaccinated mice had IFNγ-positive $CD8^+$ cells in the range of 0.017% to 0.22% for cells stimulated with *Ebola* peptide $NP_{279-288}$ (FIG. **6**). For control mice, the number of IFNγ-positive $CD8^+$ cells was significantly lower, ranging from 0.00513% to 0.00794%. No IFNγ-positive $CD8^+$ cells were detected for cells stimulated with *Ebola* peptide $VP40_{171-180}$ or $GP_{161-169}$ (data not shown).

[0143] Protective efficacy of *Ebola*ΔVP30 virus in mice. To assess the protective efficacy of the *Ebola*ΔVP30 virus, two groups of 4-week-old mice were intraperitoneally immunized, then subjected to lethal challenge with mouse-adapted *Ebola virus*. 'Group 1' mice were immunized three times at three-week intervals with 10^7 FFU of non-purified *Ebola*ΔVP30 virus (i.e., virus harvested from cell culture supernatant); eight control mice were inoculated in the same manner with 2% FCS-MEM. Mice from this group were challenged seven weeks after the last immunization with 1000 MLD_{50} of mouse-adapted *Ebola virus,* which consistently kills mice (Bray et al., 1998; Ebihara et al., 2006). 'Group 2' mice were immunized twice (with a three-week interval) with 10^6 FFU of purified *Ebola*ΔVP30 virus; ten

control mice were similarly inoculated with PBS. Mice from 'Group 2' were challenged eight weeks after the last immunization with 1000 MLD_{50} of mouse-adapted *Ebola virus*. No signs of disease or illness were seen in mice vaccinated with purified or non-purified *Ebola*ΔVP30 virus, whereas control mice from both groups began showing signs of sickness (e.g., ruffled fur) along with weight loss on day 3 post-challenge. By day 7 post-challenge, all control mice had succumbed to infection. By contrast, vaccinated mice from both groups showed no signs of disease, as characterized by ruffled fur and weight loss, and were fully protected against lethal challenge up to day 28, when all surviving mice were euthanized. On day 4 post-challenge, mice were sacrificed to determine viral titers in the sera. Vaccinated mice from both groups showed a 3 to 4 logic reduction in viral titers compared to their respective control mice. Taken together, these data demonstrate that the *Ebola*ΔVP30 virus efficiently protects mice against challenge with a lethal dose of mouse-adapted *Ebola virus*. Similar results were obtained in guinea pigs.

[0144] *Ebola*ΔVP30-immunized mice were completely protected from a lethal challenge with mouse-adapted *Ebola virus* and that the virus titers in sera from these mice were more than 1000-fold lower than those in control mice.

[0145] The humoral response to *Ebola virus* infection is important, as demonstrated by protection from a lethal challenge by passive transfer of antibodies to the viral glycoprotein GP (Gupta et al., 2001; Warfield et al., 2003). However, the ability of a vaccine to elicit an antibody response does not in itself correlate with protection from *Ebola virus* infection. For example, classical vaccine approaches, such as γ-irradiated *Ebola* and *Marburg viruses,* along with GP expressed in baculovirus generate a moderate antibody response; however, they fail to protect mice against a lethal challenge (Ignatyeve et al., 1996; Lupton et al., 1980; Mellquist-Riemenschneider et al., 2003). By contrast, *Ebola* and *Marburg* VLPs protect mice from a lethal challenge of *Ebola* or *Marburg virus* (Warfield et al., 2003; Warfield et al., 2004; Warfield et al., 2005), and not only elicit a humoral response, but also induce a $CD8^+$ T-cell response, highlighting the importance of the latter response for protection against a lethal challenge of *Ebola virus* (Warfield et al., 2005). Similarly, in non-human primates (NHPs), full protection from a lethal challenge appears to depend on both the humoral response and a $CD8^+$ cellular response (Sullivan et al., 2000). Vaccine candidates that protect NHPs from a lethal *Ebola virus* challenge, such as recombinant vesicular stomatitis virus (VSV) (Jones et al., 2005) and adenovirus (Sullivan et al., 2000), induce a $CD8^+$ T-cell response in NHPs, albeit to varying degrees (Jones et al., 2005; Sullivan et al., 2000). The *Ebola*ΔVP30 virus induced both humoral and $CD8^+$ T-cell (specific for an *Ebola* NP epitope) responses, although the extent of the latter responses varied among animals. Whether this $CD8^+$ T-cell response is sufficient to provide protection to NHPs from a lethal *Ebola virus* infection remains to be tested.

[0146] Although vaccine candidates such as recombinant VSV or parainfluenza virus offer protection in various animal models (Bukreyev et al., 2006; Jones et al., 2005), there are safety concerns with the use of these vaccines in humans (Bukreyev et al., 2006; Jones et al., 2005; Reed et al., 2007). Preexisting immunity to a vaccine based on recombinant adenovirus is also a concern, as is the large amount of virus (10^{10} particles) needed to confer protection in NHPs (Jones et al., 2005; Sullivan et al., 2000). *Ebola* and *Marburg* VLPs have been shown to protect mice and guinea pigs from a lethal challenge of these viruses (Warfield et al., 2004; Warfield et al., 2005). While VIPs are safe and, due to the rarity of *Ebola virus* infection, preexisting immunity to *Ebola* or *Marburg* viruses is not a concern for VLP vaccines, it is difficult to produce large quantities of VLPs from cell culture.

[0147] The biologically contained *Ebola*ΔVP30 virus is thus an ideal vaccine candidate since it combines the advantages of VLPs and vectored vaccines (i.e., safety and efficacy), yet it can be propagated to high titers in VeroVP30 cells like standard viruses (Example 1). Further studies will include testing the *Ebola*ΔVP30 virus for its protective efficacy in NHPs. In addition, shorter, single vaccination protocols will be evaluated to determine if the *Ebola*ΔVP30 virus vaccine could elicit fast and effective immunity in the event of an outbreak or bioterrorism attack. This includes evaluating the *Ebola*ΔVP30 virus as a vaccine for post-exposure treatment.

[0148] The invention will be further described in the following nonlimiting examples.

EXAMPLE 1

[0149] Nonhuman primates were vaccinated with one or two (prime and boost) doses of vaccine virus (IM or aerosol) and then challenged (heterologous challenge or homologous challenge) 4 weeks after the last dose, Some vaccinations included one of three different adjuvants. As shown by the data from Study #2, which employed 10-fold more vaccine virus than Study #1, only those animals vaccinated with virus and an adjuvant survived heterologous challenge. In Study #3, in addition to protocols that used a vaccine of the invention (3A and 3D), two other anti-*Ebola virus* vaccines currently undergoing testing (Vaccine A, intranasal administration of a vaccine having a replication competent virus; Vaccine B, intramuscular administration of a vaccine having a replication incompetent virus) were also tested. In one protocol, immunization only included Vaccine A (3G). In other immunization protocols, a combination of a vaccine of the invention and Vaccine A (3B and 3C) and a combination of Vaccine A and Vaccine B (3E and 3F) were tested. Some protocols included an adjuvant (3A-3D).

	Number of NHPs	Prim dose (route)	Boost dose (route)	Time between doses (weeks)	Challenge virus and dose (route)	Time between immunization and challenge (weeks)	Adjuvant	Survival	Notes
Study #1	4	10^7 FFU (IM)	—	4	Kikwit 10^4 FFU (IM)	4	—	100%	Viremia in 1 animal

-continued

	Number of NHPs	Prim dose (route)	Boost dose (route)	Time between doses (weeks)	Challenge virus and dose (route)	Time between immunization and challenge (weeks)	Adjuvant	Survival	Notes
	4	10^7 FFU (IM)	10^7 FFU (IM)	4	Kikwit 10^4 FFU (IM)	4	—	100%	—
	2	10^7 FFU (IM)	10^7 FFU (IM)	4	Kikwit 10^4 FFU (IM)	4	—	100%	—
Study #2	4	10^8 FFU (IM)	10^8 FFU (IM)	4	Makona 10^4 FFU (IM)	4	MF-59	100%	Viremia in 4 animals
	4	10^8 FFU (IM)	10^8 FFU (IM)	4	Makona 10^4 FFU (IM)	4	MPLA	50%	Viremia in 4 animals
	4	10^8 FFU (IM)	10^8 FFU (IM)	4	Makona 10^4 FFU (IM)	4	—	0%	Viremia in 4 animals

Vaccine virus is Zaire Ebola, strain Mayinga. Studies 1 and 2 are heterologous Zaire challenge viruses; study 3 is a homologous Zaire challenge virus. Study 1 is with non-inactivated vaccine virus. Studies 2 and 3 are with inactivated vaccine virus of the invention; Vaccine A is also inactivated.
FFU = focus-forming units;
IM = intramuscular

Study #3	Number of NHPs	Prime dose (route)	Boost dose (route)	Time between doses (weeks)	Challenge virus and dose (route)	Time between immunization and challenge (weeks)	Adjuvant (used with deltaVP30 only)	Survival	Notes
A	2	10^8 FFU (IM)	10^8 FFU (IM)	8	Mayinga 10^2 FFU (aerosol)	7	QS-21	50%	—
B	2	Vaccine A 4×10^8 FFU	10^8 FFU (IM)	8	Mayinga 10^2 FFU (aerosol)	7	QS-21	100%	—
C	2	10^8 FFU (IM)	Vaccine A 4×10^8 FFU	8	Mayinga 10^2 FFU (aerosol)	7	QS-21	50%	—
D	2	10^8 FFU (IM)	10^8 FFU (IM)	8	Mayinga 10^4 FFU (IM)	7	QS-21	100%	—
E	2	Vaccine B 5×10^{10} particles	Vaccine A 2×10^8 particles	8	Mayinga 10^2 FFU (aerosol)	7	—	0%	—
F	2	Vaccine A 2×10^8 particles	Vaccine B 5×10^{10} particles	8	Mayinga 10^2 FFU (aerosol)	7	—	50%	—
G	2	Vaccine A 4×10^8 particles	Vaccine A 4×10^8 particles	8	Mayinga 10^2 FFU (aerosol)	7	—	50%	—

Vaccine A - completed phase I clinical trial.
Vaccine B - completed phase I, II, and III clinical trials.
0.1 mg of QS-21 (quil-A) was employed in 3A-3D.

EXAMPLE 2

Exemplary Manufacturing Process

Generation of a Master Virus Seed (MVS)

[0150] Chemical transfection reagents to introduce the plasmids for the generation of the vaccine virus by the technique of reverse genetics is inefficient for VeroVP30 cells. Therefore, electroporation was performed using the Neon Transfection system. Prior to use, the Neon Transfection system was sterilized by ethylene oxide. Six microfuge tubes each containing 1×10e6 VeroVP30 cells were mixed with 10 ug total plasmid (2:1:1:2 mass ratio of pCAGGS EBOV 1, pCAGGS EBOV NP-VP35, T7, and pTM *Ebola*ΔVP30 plasmids, respectively) and electroporated in the Neon Transfection system at 1200 V with three 20 millisecond pulses. Transfected cells were seeded in each well of a 6-well plate and incubated at 37±2° C., 5±2% CO2 for 4 days before expansion in TC75 flasks.

Cell Culture and Vaccine Virus Harvest

[0151] Cell culture is initiated with Complete Medium (virus production-serum free medium [VP-SFM, Thermo Fisher Scientific]) supplemented with 1% Glutamax and

expanded into 18×10-layer Cell Factories (Nunc). The VP-SFM performed better than OptiVERO medium (InVitria) and the 10-layer Cell Factories performed better than hyper-stacks (Corning) in terms of virus production, resulting in an increase in virus titers (1-1.5 log increase in titer expressed in focus-forming units).

[0152] When cells reached 80-90% confluency, each 10-layer Cell Factory is washed three times with DPBS and then infected with the MVS at a MOI of 0.1 in VP-SFM plus 1% Glutamax. Each infected 10-layer Cell Factory was incubated for 7 days at 37±2° C., 5±2% CO_2. Following the seven day infection period, each 10-layer Cell Factory was inspected for contamination and the contents were harvested into a sterile 20 L bioprocess bag. Samples of pooled and the bulk harvest were pumped through a depth filter (1.2 μM filtration by Sartorius 2 XLG MidiCap) into a new sterile bioprocess bag.

[0153] Host genomic DNA was removed by benzonase treatment. $MgCl_2$ was added to the filtered viral harvest up to a final concentration of 2 mM $MgCl_2$. Benzonase (Millipore Sigma) was then added to a final concentration of 10 U/mL of filtered harvest and incubated at 37±2° C. for 4-6 hours. After benzonase treatment, β-Propiolactone (BPL) was added to a final concentration of 0.1% v/v and incubated at 2-8° C. for 16-18 hours.

[0154] Degraded host DNA, benzonase, BPL and other host impurities were subsequently removed by tangential flow filtration (TFF). The benzonase/BPL treated viral harvest was concentrated approximately 10× (e.g., concentrated so as not to result in precipitation), diafiltered, e.g., using 0.1 um pore size, into Dulbecco's Phosphate-Buffered Saline (DPBS) with Ca^{2+}/Mg^{2+}, then further concentrated using a sterile/closed TFF system (the TFF filter has a 0.1 um pore size, 30 cm path length, e.g., GE RTPCFP-1E-8S hollow fiber cartridge from GE Health) to ~2× the desired product concentration (e.g., 6×10^7 FFU/ml). Following diafiltation, samples of the TFF retentate and wash pools were analyzed to determine titer by ELISA and host protein. Retentate and wash samples meeting host protein specifications (e.g., ≤500 ng/ml) were pooled, centrifuged to remove any residual particulate matter, transferred to a sterile 2 L Erlenmeyer flask, and stored at 2-8° C. until product manufacturing (e.g., in DPBS).

Testing of Media and Conditions

[0155] When TC175 flasks were used to culture and infect cells, OPTIVero medium resulted in virus titers that were about 1 log unit greater than VP-SFM (e.g., 6.84 log10 FFU/ml [OPTIVero] vs. 5.75 log10 FFU/ml [VP-SFM). When a 10-tray system was used to culture and infect cells (large culture vessels for vaccine production), VP-SFM medium resulted in virus titers that were about 1 log unit greater than OPTIVero medium (e.g., 6.52 log10 FFU/ml [VP-SFM] vs. 5.52 log10 FFU/ml [OPTIVero). When VP-SFM medium was used to culture and infect cell in a 10-tray system, that system produce about 1 log unit greater titers than VP-SFM medium was used to culture and infect cells in a hyperstack (e.g., 6.69 log10 FFU/ml [10-tray system] vs. 5.58 log10 FFU/ml [hyperstack]). VP-SFM medium includes a plant hydrosylate (e.g., comprising di- and tri-plant peptides) and an iron chelator while OPTIVero medium includes recombinant human albumin, recombinant human transferrin (i.e., no plant hydrosylate or iron chelator).

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[0207] All publications, patents and patent applications are incorporated herein by reference. While in the foregoing specification, this invention has been described in relation to certain preferred embodiments thereof, and many details have been set forth for purposes of illustration, it will be apparent to those skilled in the art that the invention is susceptible to additional embodiments and that certain of the details herein may be varied considerably without departing from the basic principles of the invention.

1. A vaccine comprising an effective amount of a recombinant filovirus and one or more adjuvants, wherein the genome of the recombinant filovirus contains a deletion of one or more nucleotides in a polynucleotide sequence for a viral protein corresponding to *Ebola virus* VP30, and wherein the deletion is effective to prevent expression of a functional viral protein corresponding to *Ebola virus* VP30 upon infection of a cell with the recombinant filovirus.

2. (canceled)

3. The vaccine of claim **1** wherein the genome further comprises a nucleotide sequence encoding a prophylactic or therapeutic heterologous gene product.

4. (canceled)

5. The vaccine of claim **3** wherein the nucleotide sequence is inserted into the filovirus genome at a site other than the site of the deletion in the polynucleotide.

6. The vaccine of claim **5** wherein the nucleotide sequence is inserted between NP coding sequences and VP35 coding sequences in the filovirus genome.

7. The vaccine of claim **3** wherein the nucleotide sequence replaces or is inserted into GP/sGP sequences or a portion thereof.

8. (canceled)

9. The vaccine of claim **1** wherein the heterologous gene product comprises a heterologous filovirus glycoprotein.

10. The vaccine of claim **9** wherein the filovirus glycoprotein comprises a *Marburg virus, Ebola virus,* Sudan virus, Tai Forest virus, Reston virus, or Bundibugyo virus glycoprotein.

11-12. (canceled)

13. The vaccine of claim **1** wherein the adjuvant comprises lipopolysaccharide, squalene, an extract of *Quillaja saponaria* or saponin.

14-17. (canceled)

18. A method to immunize a mammal, comprising administering to the mammal an effective amount of the vaccine of claim **1**.

19-25. (canceled)

26. A multivalent vaccine comprising an effective amount of a recombinant filovirus, wherein the genome of the recombinant filovirus contains a first deletion in one or more nucleotides for a polynucleotide sequence for a viral protein corresponding to *Ebola virus* VP30 which deletion is effective to prevent expression of a functional viral protein corresponding to *Ebola virus* VP30 upon infection of a cell with the recombinant filovirus, and wherein the genome contains a mutation in a region that is flanked by NP coding sequences and VP35 coding sequences, a mutation in GP/sGP coding sequences, and/or an insertion within 1,000 nucleotides of the first deletion site or at the first deletion site, or a combination thereof, wherein the genome encodes one or more filovirus glycoproteins, wherein the mutation in the region that is flanked by NP coding sequences and VP35 coding sequences comprises an insertion of a nucleotide sequence encoding a prophylactic or therapeutic heterologous gene product and optionally also a deletion of one or more nucleotides in the region that flanks the NP coding sequences and VP35 coding sequences, wherein the mutation in the GP/sGP coding sequences comprises an insertion of a nucleotide sequence encoding a prophylactic or therapeutic heterologous gene product and optionally also a deletion of one or more nucleotides in the GP/sGP coding sequences, or wherein the insertion that is within 1,000 nucleotides of or at the first deletion site encodes a prophylactic or therapeutic heterologous gene product.

27-29. (canceled)

30. The vaccine of claim **26** wherein the gene product comprises *Ebola* NP, *Ebola* VP40, *Ebola* VP35, *Marburg* NP, *Marburg* NP VP40, *Marburg* NP VP35, *Plasmodium* circumsporozoite protein (CSP), *Plasmodium* apical membrane antigen (AMA), *Plasmodium* rhoptry neck protein 2 (RON2), *Plasmodium* RH5, *Marburg* GP, flavivirus membrane protein, flavivirus envelope protein, or a bunyavirus glycoprotein precursor (GPC) protein.

31-39. (canceled)

40. A method to immunize a mammal, comprising administering to the mammal an effective amount of the vaccine of claim **26**.

41. A method of manufacturing recombinant filovirus, comprising:

providing supernatant from mammalian cells expressing a recombinant filovirus genome that are cultured in serum free medium so as to result in progeny filovirus, wherein the genome of the recombinant filovirus contains a deletion of one or more nucleotides in a polynucleotide sequence for a viral protein corresponding to *Ebola virus* VP30, and wherein the deletion is effective to prevent expression of a functional viral protein corresponding to *Ebola virus* VP30 upon infection of a cell with the recombinant filovirus, and wherein the mammalian cells express a viral protein corresponding to *Ebola virus* VP30;

contacting the supernatant with a DNase and a virus inactivating agent, thereby providing an inactivated viral preparation; and

concentrating the inactivated viral preparation.

42. The method of claim **41** wherein the cells are Vero cells.

43. The method of claim **41** wherein the collected supernatant is filtered before contact with the DNase or the viral inactivating agent.

44. The method of claim **43** wherein the collected supernatant is subjected to filtration with a 0.5 to 5 micron filter or a 1 to 5 micron filter.

45. The method of claim **41** wherein the inactivated viral preparation is subjected to filtration.

46. The method of claim **45** wherein the filtration of the inactivated viral preparation is through a 0.01 to 1 micron filter or a 0.05 to 0.25 micron filter.

47. (canceled)

48. The method of claim **41** further comprising combining the inactivated viral preparation and one or more adjuvants.

49. A composition produced by the method of claim **40**.

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