

Table S1. List of ZIKV Genbank accession numbers of sequences used in phylogenetic analysis.

| Name | Genbank Accession # | Name | Genbank Accession # |
|-------------------------------|----------------------------|-------------------|----------------------------|
| Brazil/2015 | KU321639 | Honduras/2015 | KX694534 |
| Brazil/2015 | KU527068 | Honduras/2016 | KX262887 |
| Brazil/2015 | KX280026 | Malaysia/1966 | KX377336 |
| Brazil/2015 | KX520666 | Martinique/2015 | KU647676 |
| Brazil/2016 | KU926310 | Mexico/2015 | KX247632 |
| Brazil/2016 | KU926309 | Mexico/2016 | KX446951 |
| Cambodia/2010 | KU955593 | Micronesia/2007 | EU545988 |
| Canada/2013 | KF993678 | Nigeria/1968 | KU963574 |
| Central African Republic/1968 | KF383115 | Panama/2015 | KX156775 |
| Central African Republic/1976 | KF268948 | Panama/2015 | KX198135 |
| Central African Republic/1979 | KF268950 | Phillippines/2012 | KU681082 |
| Central African Republic/1980 | KF268949 | Puerto Rico/2015 | KX377337 |
| China/2016 | KU761564 | Samoa/2016 | KX185891 |
| Columbia/2015 | KX548902 | Senegal/1968 | KF383116 |
| Columbia/2016 | KX247646 | Senegal/1984 | HQ234501 |
| Dominican Republic/2016 | KU853012 | Senegal/1997 | KF383117 |
| Ecuador/2016 | KX879603 | Senegal/2001 | KF383119 |
| French Guiana/2015 | KU758877 | Singapore/2016 | KX813683 |
| French Polynesia/2013 | KJ776791 | Suriname/2015 | KU312312 |
| French Polynesia/2014 | KX447511 | Suriname/2016 | KU937936 |
| Guadeloupe/2016 | KX673530 | Thailand/2014 | KU680181 |
| Guatemala/2015 | KU501217 | Tonga/2016 | KX806557 |
| Guatemala/2016 | KU870645 | Uganda/1947 | KU955594 |
| Haiti/2014 | KU509998 | USA/2016 | KX842449 |
| Haiti/2016 | KX051563 | Venezuela/2016 | KX702400 |

Table S2. Source host, isolation and passage history of ZIKV strains used in this study

| Lineage | Strain | Source Host | Year | Location | Passage History | Genbank Accession |
|----------------|---------------|--------------------|-------------|---------------------|---|--------------------------|
| African | MR 766 | Rhesus monkey | 1947 | Uganda | 146x SM ^a , 1x C6/36 ^b , 1x Vero ^c | HQ234498.1 |
| Asian | PLCal_ZV | Human | 2013 | Canada/ Thailand | 4x Vero | KF993678 |
| Asian | PRVABC59 | Human | 2015 | Puerto Rico | 3x Vero | KU501215.1 |
| Asian | HS-2015-BA-01 | Human | 2015 | Brazil | 3x C6/36, 1x Vero | KX520666 |

^aSM = suckling mouse;

^bC6/36 = *Aedes albopictus* cells;

^cVero = African green monkey kidney cells.

Table S3. Percent amino acid identity matrix of the ZIKV isolates used in this study.

| | Uganda/1947 | Canada/2013 | Puerto Rico/2015 | Brazil/2015 |
|-------------------------|--------------------|--------------------|-------------------------|--------------------|
| Uganda/1947 | 100 | 97.01 | 96.90 | 98.86 |
| Canada/2013 | 97.01 | 100 | 99.76 | 99.62 |
| Puerto Rico/2015 | 96.90 | 99.76 | 100 | 99.71 |
| Brazil/2015 | 98.86 | 99.62 | 99.71 | 100 |

Figure S1

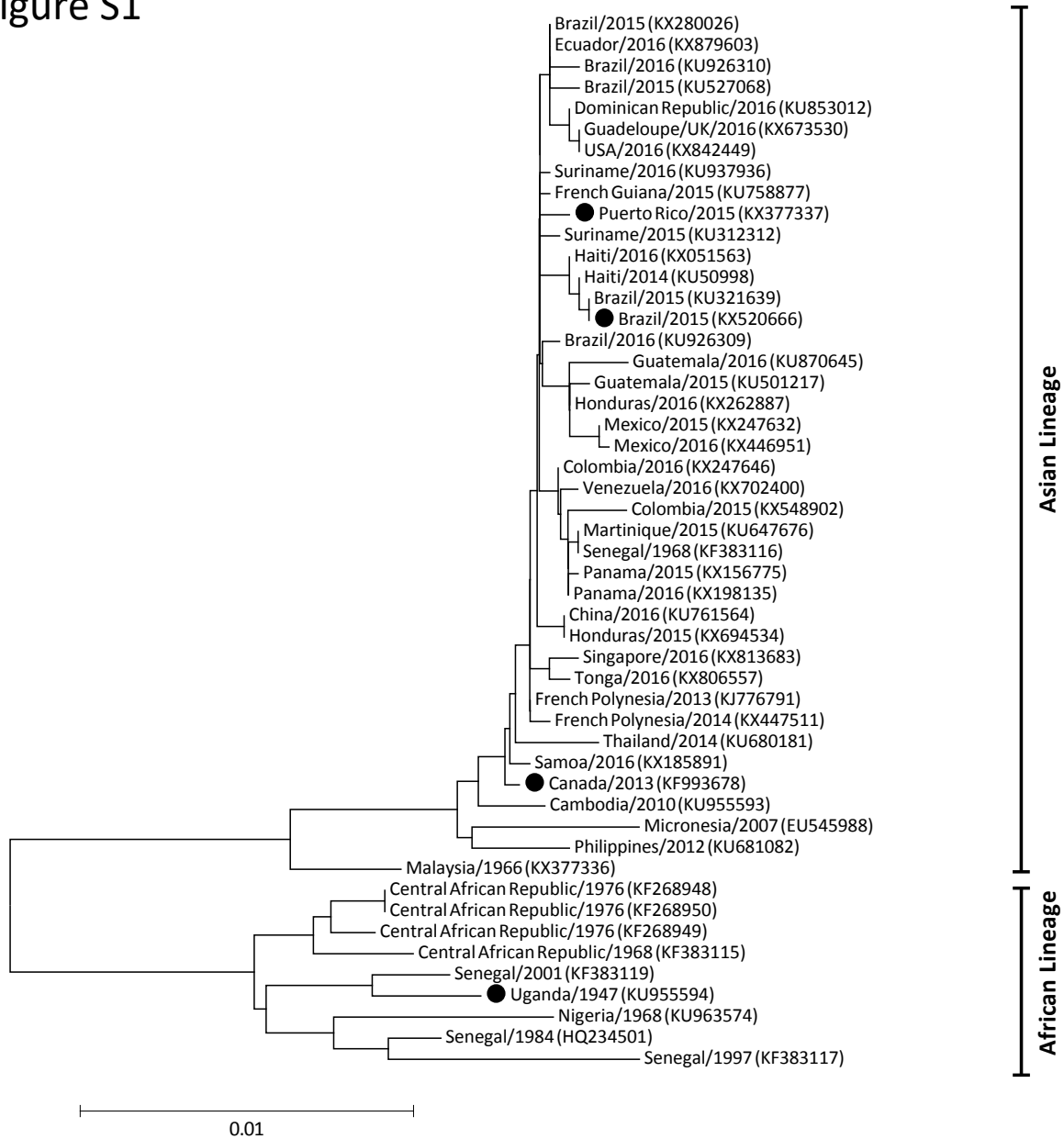


Figure S1. Phylogenetic analysis of selected ZIKV strains. Neighbor joining phylogenetic tree showing the position of the viral isolates used in this study (indicated by black circles) in the global ZIKV diversity. African and Asian lineages are indicated as well as country of origin, year of isolation, and GenBank accession number.

Figure S2

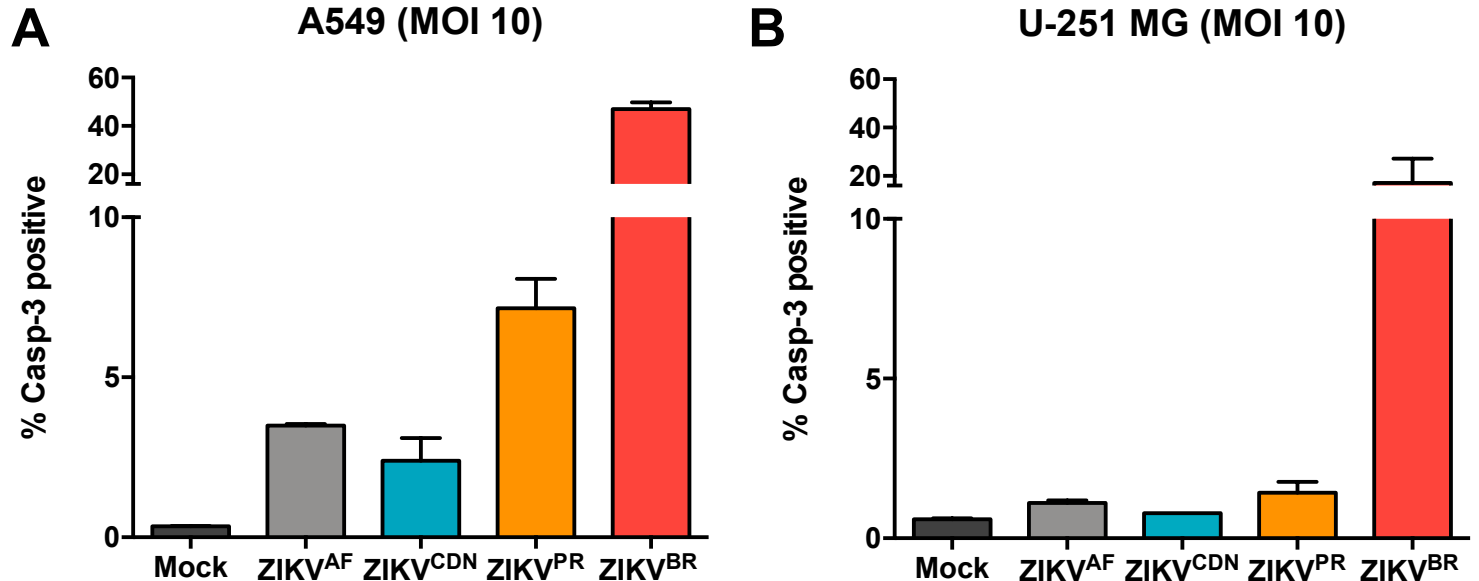


Figure S2. ZIKV isolates differentially induce apoptosis. (A) A549 cells and (B) U-251 MG cells were infected with ZIKV at MOI = 10 and 24 h post-infection the percentage of cells positive for cleaved caspase-3 was determined by flow cytometry. Values represent mean \pm SD of two independent experiments.

Figure S3

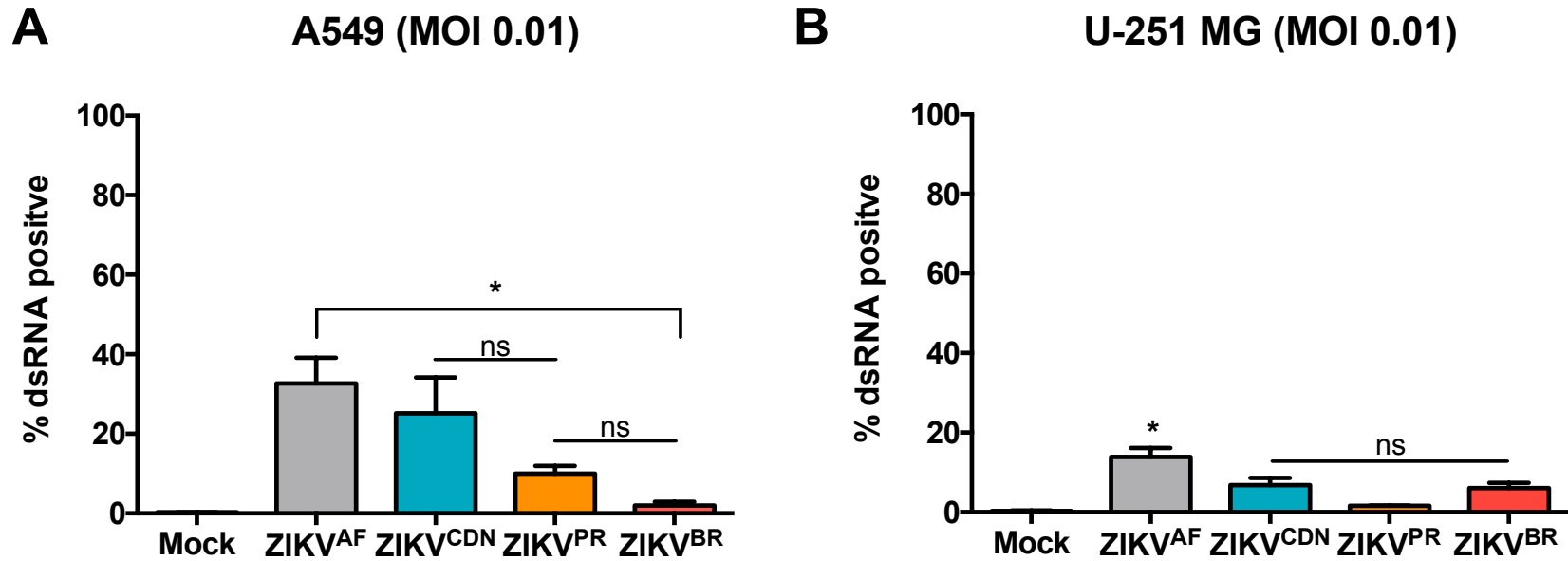


Figure S3. ZIKV isolates differ in infectivity at low MOI. (A) A549 cells and (B) U-251 MG cells were infected with ZIKV at MOI = 0.01 and 72 h post-infection the percentage of dsRNA-positive cells was determined by flow cytometry. Values represent mean \pm SEM of at least three independent experiments. Asterisks indicate significant differences in % infected cells: ** $p < 0.01$, *** $p < 0.001$.

File S1: Amino acid sequence alignment of the ZIKV isolates used in this study.

| | ↗ Capsid | |
|------------------|--|-----|
| Uganda/1947 | MKNPKKKSGGFRIVNMLKRGVARVNPLGGLKRLPAGLLLGHPIRMVLAILAFLRFTA | 60 |
| Canada/2013 |S·F..... | 60 |
| Puerto Rico/2015 |S·F..... | 60 |
| Brazil/2015 |S·F..... | 60 |
| Uganda/1947 | PSLGLINRWGSVGGKEAMEI IKKFKKDLAAMLRIINARKERKRRGADTSIGIIGLLTTA | 120 |
| Canada/2013 |K·T·V·V..... | 120 |
| Puerto Rico/2015 |T.....K·V·V..... | 120 |
| Brazil/2015 |K·V·V..... | 120 |
| | ↗ pre M | |
| Uganda/1947 | MAAEITRRGSAYMYLDRSDAGKAISFATTLGVNKCHVQIMDLGHMCDATMSYECPLDE | 180 |
| Canada/2013 |V.....E·P·M·YI..... | 180 |
| Puerto Rico/2015 |V.....N·E·P·M·YI..... | 180 |
| Brazil/2015 |V.....N·E·P·M·YI..... | 180 |
| Uganda/1947 | GVEPDDVDCWCNTTSTWVVYGTCHHHKGEARRSRRAVTLPSHSTRKLQTRSQTWLESREY | 240 |
| Canada/2013 | | 240 |
| Puerto Rico/2015 | | 240 |
| Brazil/2015 | | 240 |
| | ↗ E protein | |
| Uganda/1947 | TKHLIKVENWIFRNPGFALVAVAIAWLLGSSTSQKVIYLVMIILLIAPAYSIRCI | 300 |
| Canada/2013 |R·A·A..... | 300 |
| Puerto Rico/2015 |R·A·A..... | 300 |
| Brazil/2015 |R·A·A..... | 300 |
| Uganda/1947 | FVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMAS | 360 |
| Canada/2013 | | 360 |
| Puerto Rico/2015 | | 360 |
| Brazil/2015 |I..... | 360 |
| Uganda/1947 | DSRCPTQGEAYLDKQSDTYVCKRTLVDNRGNGCGLFGKGLVTCAKFTCSKMTGKSI | 420 |
| Canada/2013 |A..... | 420 |
| Puerto Rico/2015 |A..... | 420 |
| Brazil/2015 |A..... | 420 |
| Uganda/1947 | QPENLEYRIMLSVHGSQHSGMI----GYETDENRAKVEVTPNSPRAEATLGGFGSLGLDC | 476 |
| Canada/2013 |VNDT·H·I..... | 480 |
| Puerto Rico/2015 |VNDT·H·I..... | 480 |
| Brazil/2015 |VNDT·H·I..... | 480 |
| Uganda/1947 | EPRTGLDFSDLYLTMNNKHVLVHKEWFHDIPLPWHAGADTGTTPHWNKEALVEFKDAHA | 536 |
| Canada/2013 | | 540 |
| Puerto Rico/2015 | | 540 |
| Brazil/2015 | | 540 |
| Uganda/1947 | KRQTVVVLGSQEGAVHTALAGALEAEMDGAKGLFSGHLKCRKMDKRLKGVSYSLCTA | 596 |
| Canada/2013 |R·S..... | 600 |
| Puerto Rico/2015 |R·S..... | 600 |
| Brazil/2015 |R·S..... | 600 |
| Uganda/1947 | AFTFTKVPAETLHGTVTVEVQYAGTDGPKIPVQMAVDMQTLTPVGRGLITANPVITESTE | 656 |
| Canada/2013 |I.....V·A..... | 660 |
| Puerto Rico/2015 |I.....V·A..... | 660 |
| Brazil/2015 |I.....V·A..... | 660 |
| Uganda/1947 | NSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSSTIGKAFEATVRGAKRMAVLGDTAWD | 716 |
| Canada/2013 |E..... | 720 |
| Puerto Rico/2015 |E..... | 720 |
| Brazil/2015 |E..... | 720 |
| Uganda/1947 | FGSVGGVFNSLGGKIHQIFGAAFKSLFSGMSWFSQILIGTLLVWLGLNKNKSISLTCLA | 776 |
| Canada/2013 |AL.....M·M..... | 780 |
| Puerto Rico/2015 |AL.....M·M..... | 780 |
| Brazil/2015 |AL.....M·M..... | 780 |
| | ↗ NS1 | |
| Uganda/1947 | LGGVMIFLSTAVSADVGCSDVFSKKEVTRCGTGVFIYNDVEAWRDRYKHPDPRRLAAAV | 836 |
| Canada/2013 |L.....V..... | 840 |
| Puerto Rico/2015 |L.....V..... | 840 |
| Brazil/2015 |L.....V..... | 840 |

| | | |
|------------------|--|------|
| Uganda/1947 | KQAWEEGICGISSVSRMENIMWKSVEGELNAILEENGVQLTVVVGSVKNPMWRGPQRLPV | 896 |
| Canada/2013 |D.....R..... | 900 |
| Puerto Rico/2015 |D.....R.....G..... | 900 |
| Brazil/2015 |D.....R..... | 900 |
| Uganda/1947 | PVNELPHGWKAWGKSYFVRAAKTNNNSFVVDGDTLKECPLHRAWNSFLVEDHGFGVFHTS | 956 |
| Canada/2013 |K..... | 960 |
| Puerto Rico/2015 |K..... | 960 |
| Brazil/2015 |H.....K..... | 960 |
| Uganda/1947 | VWLKVEDYSLECDPAVIGTAVKGREAAHSDLGWIESEKNDTWRLKRAHLIEMKTCEWP | 1016 |
| Canada/2013 |K.....V..... | 1020 |
| Puerto Rico/2015 |K.....V..... | 1020 |
| Brazil/2015 |K.....V..... | 1020 |
| Uganda/1947 | KSHTLWTDGVEESDLIIPKSLAGPLSHHNTREGYRTQVKGPWHSEELEIRFEECPGTKVY | 1076 |
| Canada/2013 |I.....M.....H..... | 1080 |
| Puerto Rico/2015 |I.....M.....H..... | 1080 |
| Brazil/2015 |I.....M.....H..... | 1080 |
| Uganda/1947 | VEETCGTRGPSLRSTTASGRVIEEWCCRECTMPPLSFRAKDGCWYGMEIRPRKEPESNLV | 1136 |
| Canada/2013 | | 1140 |
| Puerto Rico/2015 | | 1140 |
| Brazil/2015 | | 1140 |
| | ↳ NS2A | |
| Uganda/1947 | RSMVTA [↓] GS [↓] TDHMDH [↓] FL [↓] SLGVLVILLMVQEG [↓] LKKRMTKIIMSTSMALV [↓] VVMILGGFSMSDL | 1196 |
| Canada/2013 |I.....A..... | 1200 |
| Puerto Rico/2015 |I.....A..... | 1200 |
| Brazil/2015 |I.....A..... | 1200 |
| Uganda/1947 | AKLVILMGATFAEMNTGGDVAHLALVA [↓] AFKVRPALLVSFIFRANWTPRESMLLALASCLL | 1256 |
| Canada/2013 |A.....I..... | 1260 |
| Puerto Rico/2015 |A.....I..... | 1260 |
| Brazil/2015 |A.....I..... | 1260 |
| Uganda/1947 | QTAISALEGDLMLVINGFALAWLAIRAMAVPRTDNIALPILAALTPLARGTLLVAWRAGL | 1316 |
| Canada/2013 |V.....T.A..... | 1320 |
| Puerto Rico/2015 |V.....T.A..... | 1320 |
| Brazil/2015 |V.....T.A..... | 1320 |
| | ↳ NS2B | |
| Uganda/1947 | ATCGGIMLLSLKKGKSVKKNLFFVMALGLTAVRVVDPINVVGLLLLTRSGKRS [↓] WPPSEVL | 1376 |
| Canada/2013 |F.....L..... | 1380 |
| Puerto Rico/2015 |F.....L..... | 1380 |
| Brazil/2015 |F.....L..... | 1380 |
| Uganda/1947 | TAVGLICALAGGFAKADIEMAGPMAAVGLLIVSYVVS [↓] GKSVDMYIERAGDITWEKDAEVT | 1436 |
| Canada/2013 | | 1440 |
| Puerto Rico/2015 | | 1440 |
| Brazil/2015 | | 1440 |
| Uganda/1947 | GNSPRLDVALDESGDFSLVEEDGPPMREIILKVVLMAICGMNPIAIPFAAGAWYVYK [↓] TG | 1496 |
| Canada/2013 |D.....T..... | 1500 |
| Puerto Rico/2015 |D.....T..... | 1500 |
| Brazil/2015 |D.....T..... | 1500 |
| | ↳ NS3 | |
| Uganda/1947 | KRS [↓] GALWDVPAPKEVKKGETTDGVYRVMTRRLLGSTQVGVGMQEGVFHTMWHVTKGAAL | 1556 |
| Canada/2013 |S..... | 1560 |
| Puerto Rico/2015 |S..... | 1560 |
| Brazil/2015 |S..... | 1560 |
| Uganda/1947 | RSGEGRLD [↓] DPYWDV [↓] KQDLVSYCGPWKLDAAWDGLSEVQLLAVPPGERARNIQTLP [↓] GIFKT | 1616 |
| Canada/2013 |H..... | 1620 |
| Puerto Rico/2015 |H..... | 1620 |
| Brazil/2015 |H..... | 1620 |
| Uganda/1947 | KDGDIGAVALDYPAGTSGSPILDKCGRVIGLYGNGVVIKNGSYVSAITQ [↓] GKREEETPVEC | 1676 |
| Canada/2013 |R..... | 1680 |
| Puerto Rico/2015 |R..... | 1680 |
| Brazil/2015 |R..... | 1680 |

| | | |
|------------------|-------------------------------|------|
| Uganda/1947 | FEPSMLKKKQLTVLDDLHPGAGKTRRVLP | 1736 |
| Canada/2013 |T..... | 1740 |
| Puerto Rico/2015 |T..... | 1740 |
| Brazil/2015 |T..... | 1740 |
| Uganda/1947 | LPVRYMTTAVNVTHSGTEIVDLMCHATF | 1796 |
| Canada/2013 | | 1800 |
| Puerto Rico/2015 | | 1800 |
| Brazil/2015 | | 1800 |
| Uganda/1947 | GYISTRVEMGEAAAIFMTATPPGTRDA | 1856 |
| Canada/2013 | | 1860 |
| Puerto Rico/2015 | | 1860 |
| Brazil/2015 |Y..... | 1860 |
| Uganda/1947 | TVWVFPVSVRNGNEIAACLTKAGKRVI | 1916 |
| Canada/2013 |H.....V..... | 1920 |
| Puerto Rico/2015 |H.....V..... | 1920 |
| Brazil/2015 |H.....V..... | 1920 |
| Uganda/1947 | KADRVIDSRCLKPVILDGERVILAGPMP | 1976 |
| Canada/2013 |L..... | 1980 |
| Puerto Rico/2015 |L..... | 1980 |
| Brazil/2015 |L..... | 1980 |
| Uganda/1947 | ETDEGHAHWLEARMLLDNIYLQDGLI | 2036 |
| Canada/2013 |D..... | 2040 |
| Puerto Rico/2015 |D..... | 2040 |
| Brazil/2015 |D..... | 2040 |
| Uganda/1947 | GDLPVWLAYQVASAGITYTDRRWC | 2096 |
| Canada/2013 |R..... | 2100 |
| Puerto Rico/2015 |RH..... | 2100 |
| Brazil/2015 |RH..... | 2100 |
| Uganda/1947 | VCS | 2156 |
| Canada/2013 |F..... | 2160 |
| Puerto Rico/2015 |F..... | 2160 |
| Brazil/2015 |F..... | 2160 |
| Uganda/1947 | KAAAAQLPETLETIMLLGLLGTVSLGI | 2216 |
| Canada/2013 | | 2220 |
| Puerto Rico/2015 | | 2220 |
| Brazil/2015 | | 2220 |
| Uganda/1947 | PARIACVLIVVFLLLVLIPEPEKQRS | 2276 |
| Canada/2013 |S..... | 2280 |
| Puerto Rico/2015 |S..... | 2280 |
| Brazil/2015 |S..... | 2280 |
| Uganda/1947 | DIAHLMGRREGATMGFSMDIDLRPAS | 2336 |
| Canada/2013 |LS.....I.....F..... | 2340 |
| Puerto Rico/2015 |LS.....I.....F..... | 2340 |
| Brazil/2015 |LS.....I.....F..... | 2340 |
| Uganda/1947 | TQAGVLFMGKMPFYAWDLGVPLLMGC | 2396 |
| Canada/2013 |F.....I..... | 2400 |
| Puerto Rico/2015 |F.....I..... | 2400 |
| Brazil/2015 |F.....I..... | 2400 |
| Uganda/1947 | ARAAQKRTAAGIMKNPVVDGIVVTD | 2456 |
| Canada/2013 |V.....I.....S..... | 2460 |
| Puerto Rico/2015 |V.....I.....S..... | 2460 |
| Brazil/2015 |M.....V.....I.....S..... | 2460 |
| Uganda/1947 | WGEAGALITAAATSTLWEGSPNKYWN | 2516 |
| Canada/2013 | | 2520 |
| Puerto Rico/2015 | | 2520 |
| Brazil/2015 | | 2520 |

→ NS4A

→ protein 2K

→ NS4B

→NS5

| | | |
|------------------|--|------|
| Uganda/1947 | GGGTGETLGEKWKARLNQMSALEFYYSYKSGITEVCREEARRALKDGVATGGHAVSRGSA | 2576 |
| Canada/2013 | | 2580 |
| Puerto_Rico/2015 | | 2580 |
| Brazil/2015 | | 2580 |
| Uganda/1947 | KLRWLVERGYLQPYGKVVDLGCGRGWSYYAATIRKVQEVRYTKGGPGHEEPMLVQSYG | 2636 |
| Canada/2013 |I.....K..... | 2640 |
| Puerto_Rico/2015 |I.....V.....K.....V..... | 2640 |
| Brazil/2015 |I.....K.....V..... | 2640 |
| Uganda/1947 | WNIVRLKSGVDVFMMAAEPDITLLCDIGESSSSPEVEETRILRVLSMVGDWLEKRPGAF | 2696 |
| Canada/2013 |A..... | 2700 |
| Puerto_Rico/2015 |A..... | 2700 |
| Brazil/2015 |A..... | 2700 |
| Uganda/1947 | IKVLCPYTSTMMETMERLQRRHGGGLVVRPLSRNSTHEMYWVSGAKSNIKSVSTTSQLL | 2756 |
| Canada/2013 |L.....Y.....T..... | 2760 |
| Puerto_Rico/2015 |L.....Y.....T..... | 2760 |
| Brazil/2015 |L.....Y.....T..... | 2760 |
| Uganda/1947 | LGRMDGPRRPVKYEEVDNLGSGTRAVASCAEAPNMKIIIGRIERIRNEHAETWFLDENHP | 2816 |
| Canada/2013 |V.....N.....S.....F..... | 2820 |
| Puerto_Rico/2015 |V.....N.....S.....F..... | 2820 |
| Brazil/2015 |V.....N.....S.....F..... | 2820 |
| Uganda/1947 | YRTWAYHGSYEAPTQGSASSLVNGVRLLSKFPDVTGVTGIAMTDTPYQQRVFKEKV | 2876 |
| Canada/2013 |I..... | 2880 |
| Puerto_Rico/2015 |I..... | 2880 |
| Brazil/2015 |I..... | 2880 |
| Uganda/1947 | DTRVPDPQEGTRQVMNIVSSWLKELGKRKRPRVCTKEEFINKVRSNAALGAI FEEEEKW | 2936 |
| Canada/2013 |I·SM.....H..... | 2940 |
| Puerto_Rico/2015 |SM.....H..... | 2940 |
| Brazil/2015 |SM.....H..... | 2940 |
| Uganda/1947 | KTAVEAVNDPRFWALVDREREHHLRGEGCHSCVYNNMGKREKKQGEFGKAKGSRAIYMWL | 2996 |
| Canada/2013 |K.....Q..... | 3000 |
| Puerto_Rico/2015 |K.....Q..... | 3000 |
| Brazil/2015 |K.....Q..... | 3000 |
| Uganda/1947 | GARFLEFEALGFLNEDHWMGRENSSGGVEGLGLQRLGYILEEMNRAPGGKMYADDTAGWD | 3056 |
| Canada/2013 |V.....S·I·R..... | 3060 |
| Puerto_Rico/2015 |V.....S·I·R..... | 3060 |
| Brazil/2015 |V.....S·I·R..... | 3060 |
| Uganda/1947 | TRISKFDLENEALITNQMEEGHRTLALAVIKYTYQNKVVKVLRPAEGGKTVMDDIISRDQ | 3116 |
| Canada/2013 | ···R···K···A···I···K··· | 3120 |
| Puerto_Rico/2015 | ···R···K···A···I···K··· | 3120 |
| Brazil/2015 | ···R···K···A···I···K··· | 3120 |
| Uganda/1947 | RGSGQVVTYALNTFTNLVVQLIRNMEAEVLEMQLDWWLLRKEKVTWRWLQSNQWDRDKRM | 3176 |
| Canada/2013 |RS···N..... | 3180 |
| Puerto_Rico/2015 |RS···N..... | 3180 |
| Brazil/2015 |RS···N..... | 3180 |
| Uganda/1947 | AVSGDDCVVKPIDDRFAHALRFLNDMGKVRKDTQEWKPSGTGWSNWEEVPPFCSHHFNKLYL | 3236 |
| Canada/2013 |D.....H..... | 3240 |
| Puerto_Rico/2015 |D.....H..... | 3240 |
| Brazil/2015 |D.....H..... | 3240 |
| Uganda/1947 | KDGRSIVVPCRHQDELIGRARVSPGAGWSIRETACLAKSYAQMWQLLYFHRRDLRLMANA | 3296 |
| Canada/2013 | | 3300 |
| Puerto_Rico/2015 | | 3300 |
| Brazil/2015 | | 3300 |
| Uganda/1947 | ICSAVPVDWVPTGRTTWSIHGKGEWMTTEDMLMVVNRVWIEENDHMDKTPVTKWTDIPY | 3356 |
| Canada/2013 | ···S···V··· | 3360 |
| Puerto_Rico/2015 | ···S···V··· | 3360 |
| Brazil/2015 | ···S···V··· | 3360 |

| | | |
|------------------|--|------|
| Uganda/1947 | LGKREDLWCGSLIGHRPRTTWAENIKDTVNMVRRRIIGDEEKYMDYLSAQVRYLGEEGSP | 3416 |
| Canada/2013 |N..... | 3420 |
| Puerto Rico/2015 |N..... | 3420 |
| Brazil/2015 |N..... | 3420 |
| Uganda/1947 | GVL | 3419 |
| Canada/2013 | ... | 3423 |
| Puerto Rico/2015 | ... | 3423 |
| Brazil/2015 | ... | 3423 |