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Interim Report on SNP analysis and forensic microarray probe design for South American hemorrhagic fever viruses, tick-borne encephalitis virus, henipaviruses, Old World Arenaviruses, filoviruses, Crimean-Congo hemorrhagic fever viruses, Rift Valley fever viruses and Japanese encephalitis viruses

C. Jaing, S. Gardner

June 12, 2012

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Interim Report on SNP analysis and forensic microarray probe design for South American hemorrhagic fever viruses, tick-borne encephalitis viruses, henipaviruses, Old World Arenaviruses, filoviruses, Crimean-Congo hemorrhagic fever viruses, Rift Valley fever viruses and Japanese encephalitis viruses

Project Title: Forensic TaqMan and Microarray Assays for Viral Genotyping

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Introduction

The goal of this project is to develop forensic genotyping assays for select agent viruses, enhancing the current capabilities for the viral bioforensics and law enforcement community. We used a multipronged approach combining bioinformatics analysis, PCR-enriched samples, microarrays and TaqMan assays to develop high resolution and cost effective genotyping methods for strain level forensic discrimination of viruses. We have leveraged substantial experience and efficiency gained through year 1 on software development, SNP discovery, TaqMan signature design and phylogenetic signature mapping to scale up the development of forensics signatures in year 2. In this report, we have summarized the whole genome wide SNP analysis and microarray probe design for forensics characterization of South American hemorrhagic fever viruses, tick-borne encephalitis viruses and henipaviruses, Old World Arenaviruses, filoviruses, Crimean-Congo hemorrhagic fever virus, Rift Valley fever virus and Japanese encephalitis virus.

Methods

SNP analyses were performed on all available full genomes or segments using kSNP. kSNP is a bioinformatics tool for sequence comparison and can scale to hundreds of bacterial or viral genomes, and can be used for finished and/or draft genomes available as unassembled contigs [1]. The method is fast to compute, finding SNPs and building a SNP phylogeny in seconds to hours depending on input. The approach can handle as input hundreds of megabases to gigabases of sequence in a single run. k=13 identified more SNP alleles than k=25. Fewer SNPs were found with the larger k because a longer length of conserved sequence surrounding the SNP is required. With these divergent viruses, shorter k means that SNPs in closer proximity to one another can be found, thus reducing the stringency for conservation surrounding a SNP. A value of k=13 for viruses should provide better resolution of unsequenced novel isolates than k=25, so all results reported below are for k=13, unless otherwise indicated. The results are summarized in Table 1.

Phylogenetic trees were created using:

1) Multiple sequence alignments (MSA), or full genome multiple sequence alignment with MUSCLE (http://www.drive5.com/muscle/; [2]) followed by maximum likelihood with 1000 bootstrap replicates using RAxML (http://icwww.epfl.ch/~stamatak/index-Dateien/software/RAxML-Manual.7.0.4.pdf ; [3])
2) SNP Hamming distance, calculated as the number of SNPs that differ between each pair of genomes and trees built using the neighbor method in PHYLIP
3) SNP RAxML, analyzing just the SNP alleles concatentated into a string for each genome, to make a SNP alignment from multiple genomes that can be treated as though it were an MSA, then using RAxML to create a maximum likelihood tree with 1000 bootstrap replicates from this SNP alignment.

Branch specific SNP allele counts are plotted on nodes, strain/genome specific allele counts are given in brackets after strain name. For each target set, the tree (full genome MSA, SNP distance, or SNP RAxML) that results in the most SNPs mapping to the tree is shown (that is, the tree that yields the fewest homoplastic SNPs).

Microarray probes were designed for every SNP. Probe design strategy maximized sensitivity and specificity based on extensive prior lab testing on a NimbleGen microarray platform, where we demonstrated 100% SNP allele call rates and 99.5% accuracy (in prep, and unpublished reports for DHS). We determined that maximum sensitivity and SNP discrimination accuracy result if the SNP base is at the 13th position from the 5' end of the probe (the end farthest from the array), probes are between 32 and 40 bases long, and length varies so as to equalize hybridization free energy (ΔG) to the extent possible within the allowable length range. Probes shorter than 32 bases have high false negative rates, and longer probes are inefficient at discriminating single base mismatches. We found that ΔG is a better predictor of hybridization than T_m. Probe candidates with hybridization free energy below ΔG=-43 kcal/mol were shortened until either their ΔG exceeded -43 kcal/mol or they reached the minimum 32 bases. Probes were designed around the SNP on both the plus and minus strands, for all 4 possible SNP alleles, and all surrounding sequence variants. We design probes for both the plus and minus strand; these are not the reverse complements of one another because the SNP does not lie at the center of the probe. There are probes for each of the 4 variants on each strand, so at least 8 probes per SNP locus. In addition, any sequence variation outside of the k-mer SNP context of conserved bases is captured in multiple alternative probes for that allele, so there may be more than 8 probes per SNP locus, although for a given hybridization, only the probe variant with the best signal is used for assessing the SNP allele at the 13th position. Finally, probes are trimmed from the 3' end to remove any N's or other degenerate bases, and omitted altogether if doing so results in a probe less than 32 bases. If a probe is a subsequence of any other, only the shorter of the two is kept. If necessary to fit on the desired array format, probes can be omitted for alleles not represented in the target sequences, e.g. for biallelic SNPs half the probes can be pruned. Both full unpruned sets of probes and those reduced to the observed allele variants are provided.

Results

The viruses analyzed have between 5 and 144 full length finished and draft genomes or segments available for analysis (Table 1). We found between 182 and 10,276 SNP loci for each organism. The detailed phylogenetic trees are shown after Table 1. Branch-specific SNP allele counts are plotted on nodes, strain/genome specific allele counts are given in brackets after strain name. For each target set, the tree that results in the most SNPs mapping to the tree is shown (that is, the tree that yields the fewest homoplastic SNPs).

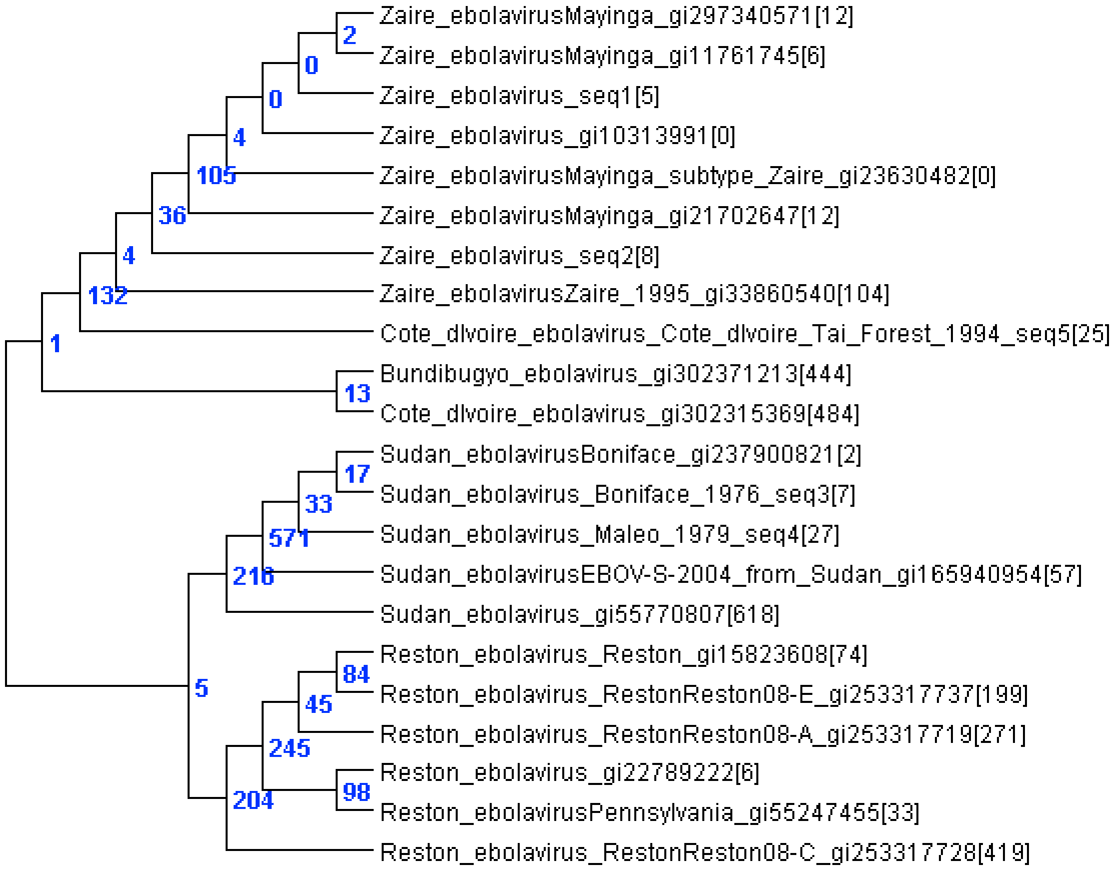
Table 1. SNP analysis results summary.

Organism	# target sequences	# SNP loci	# probes	# probes for observed alleles only	Strain resolution
Ebola	22	3083	56,113	16,163	All strains can be uniquely resolved
Marburg	31	2922	65,413	18,951	All strains can be uniquely resolved
CCHF L	31	7216	233,005	62,837	All strains can be uniquely resolved
CCHF M	49	4720	144,741	38,729	All strains can be uniquely resolved
CCHF S	56	1073	40,437	11,145	All strains can be uniquely resolved
RVF L	62	1190	39,213	12,181	All strains can be uniquely resolved
RVF M	69	897	33,025	9,888	All strains can be uniquely resolved
RVF S	89	436	17,993	5,381	Only 2 strains cannot be uniquely resolved on the basis of SNPs: Rift_Valley_fever_200803166_segmentS_gi330422537 Rift_Valley_fever_Kenya_9800523_segmentS_gi87622508
JEV	144	6759	273,205	78,468	All strains can be uniquely resolved
OW Arena L	45	7556	173,157	44,031	All strains can be uniquely resolved
OW Arena S	54	4657	120,873	30,698	There are 2 pairs of strains that cannot be uniquely resolved on the basis of SNPs: Lassa_AV_GPC_and_nucleoprotein_NP_genes_gi46373061 Lassa_AV_gi354681510 Lassa_recombinant_Josiah_segmentS_gi32390330 Lassa_segmentS_gi23343509
NW Arena S	100	5410	139,505	37,249	The following 2 pairs of strains cannot be uniquely resolved on the basis of SNP: Machupo_Carvallo_segmentS_gi22901290 Machupo_Carvallo_segmentS_gi48095765 Pichinde_gi332639 Pichinde_gi55733698
NW Arena L	42	4389	80,973	20,800	All strains can be uniquely resolved
Junin L	12	182	2397	812	All strains can be uniquely resolved
Junin S	26	660	20,341	6,100	All strains can be uniquely resolved
Machupo L	5	373	6173	1719	All strains can be uniquely resolved
Machupo S	13	614	15,309	4,336	The following 4 strains cannot be uniquely resolved on the basis of SNPs: Machupo_Carvallo_segmentS_gi22901290 Machupo_Carvallo_segmentS_gi45826501 Machupo_Carvallo_segmentS_gi48095765 Machupo_segmentS_gi34365532
Nipah	9	684	10,573	3,090	All strains can be uniquely resolved
Hendra	10	437	5,841	2,280	All strains can be uniquely resolved
TBEV	67	10,276	288,269	78,225	All strains can be uniquely resolved

Abbreviations: CCHF=Crimean-Congo hemorrhagic fever, RVF=Rift Valley fever, JEV=Japanese encephalitis virus, NW Arena=New World Arenavirus, OW Arena=Old World Arenavirus, TBEV=tick-borne encephalitis virus.

Ebola

Number_SNPs: 3083
Number_Homoplastic_SNPs from MSA tree (shown below): 97
Number_Homoplastic_SNPs from SNP Hamming distance tree: 123
Number_Homoplastic_SNPs from SNP RAxML tree: 306
All strains can be uniquely resolved on the basis of SNPs.
Number of SNP microarray probes:
56,113 probes for all alleles
16,163 probes for observed alleles only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Sudan_ebolavirus_Maleo_1979_seq4[27]
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Reston_ebolavirus_gi22789222[6]
98
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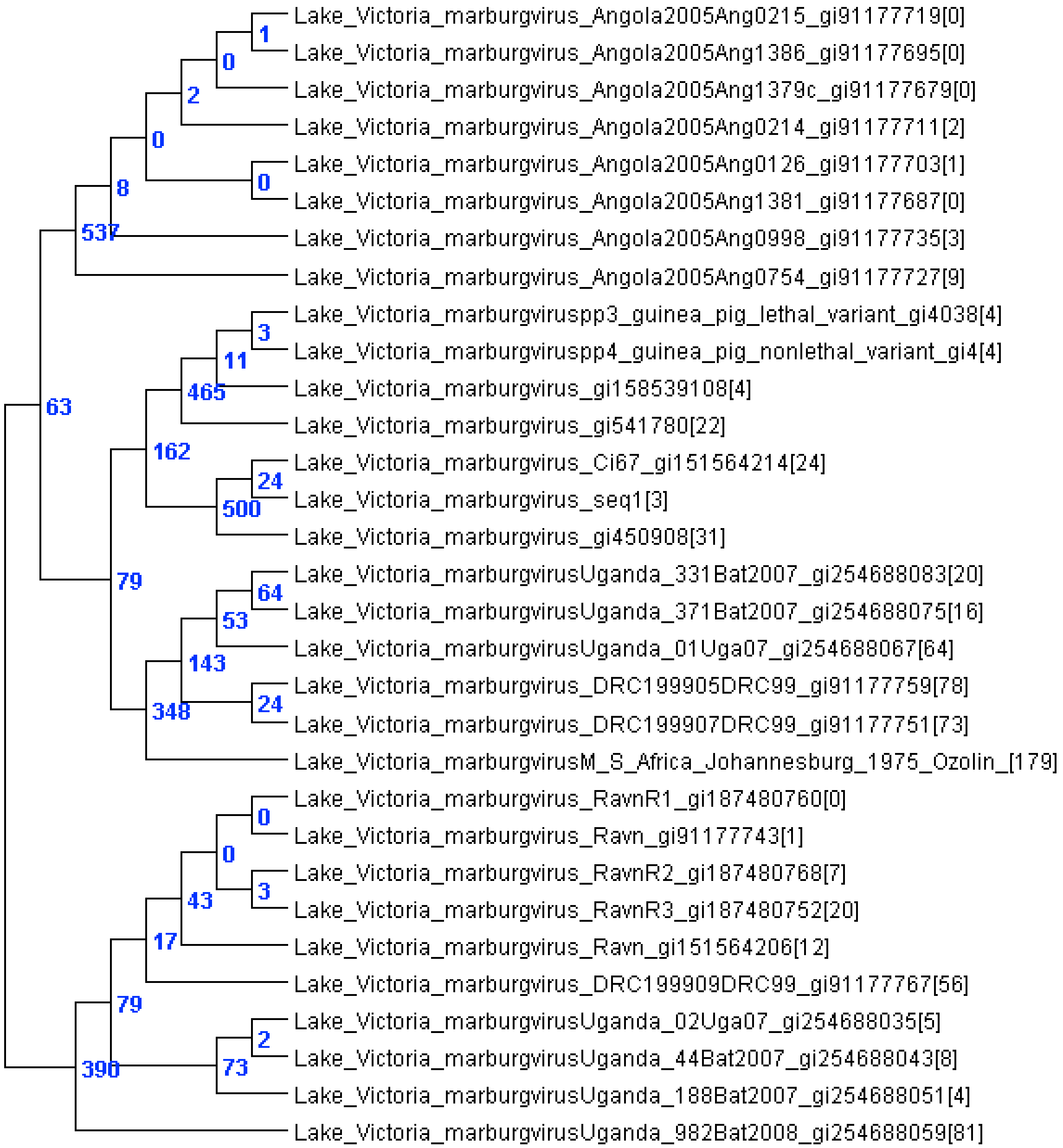
Marburg

Number_SNPs: 2922
Number_Homoplastic_SNPs from MSA tree: 1168
Number_Homoplastic_SNPs from SNP Hamming distance tree (shown below): 200
Number_Homoplastic_SNPs from SNP RAxML tree: 214
All strains can be uniquely resolved on the basis of SNPs.
Number of SNP microarray probes:
65,413 probes for all alleles
18,951 probes for observed alleles only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398
73
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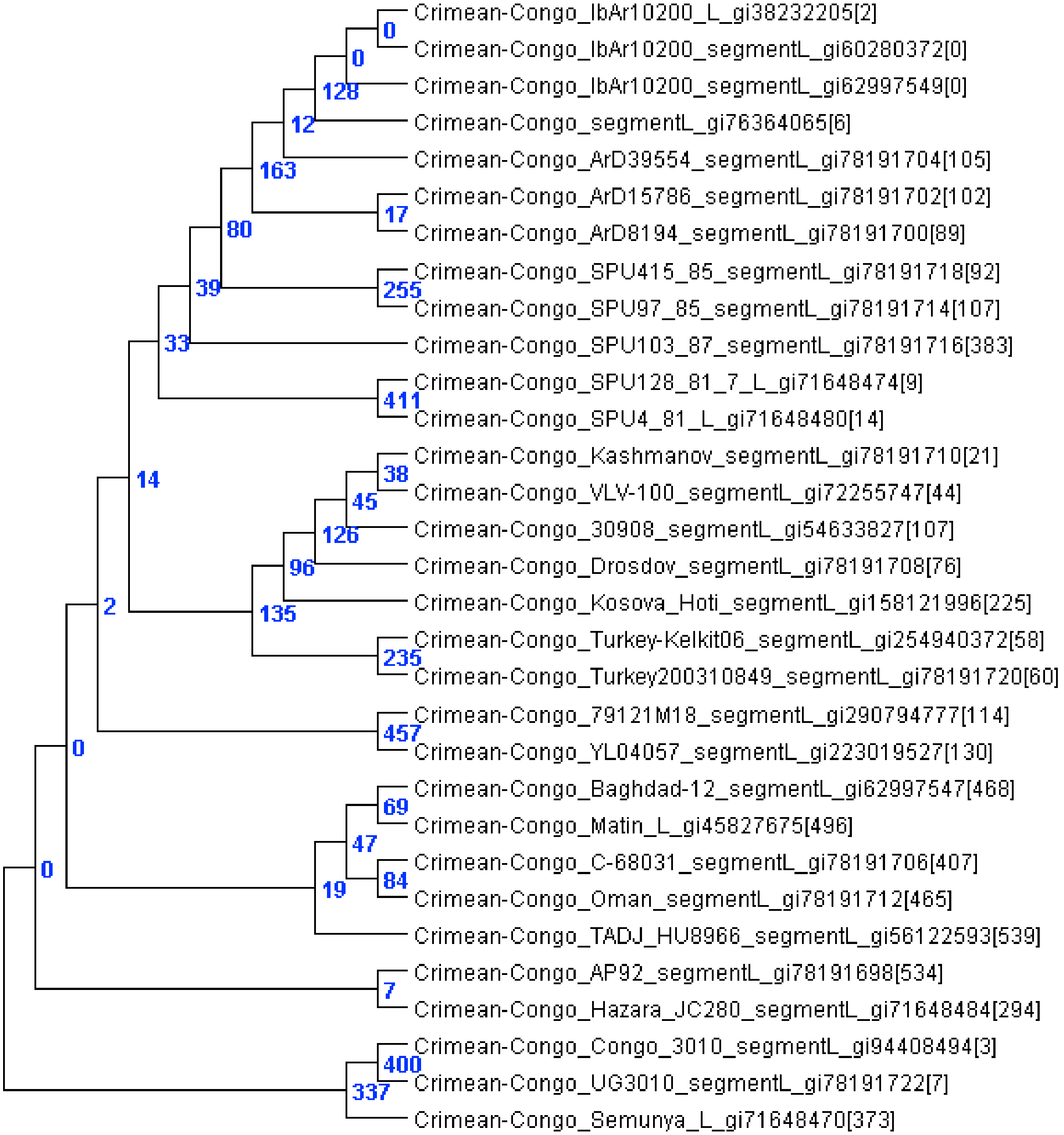
Lake_Victoria_marburgvirusUganda_982Bat2008_gi254688059[81]

CCHF L segment

Number_SNPs: 7216
Number_Homoplastic_SNPs from MSA tree: 1422
Number_Homoplastic_SNPs from SNP Hamming distance tree (shown below): 1223
Number_Homoplastic_SNPs from SNP RAxML tree: 1270
All strains can be uniquely resolved on the basis of SNPs.
Number of SNP microarray probes:
233,005 probes for all alleles
62,837 probes for observed alleles only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Crimean-Congo_ArD15786_segmentL_gi78191702[102]
17
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80
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Crimean-Congo_30908_segmentL_gi54633827[107]
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Crimean-Congo_Drosdov_segmentL_gi78191708[76]
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Crimean-Congo_YL04057_segmentL_gi223019527[130]
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400
337
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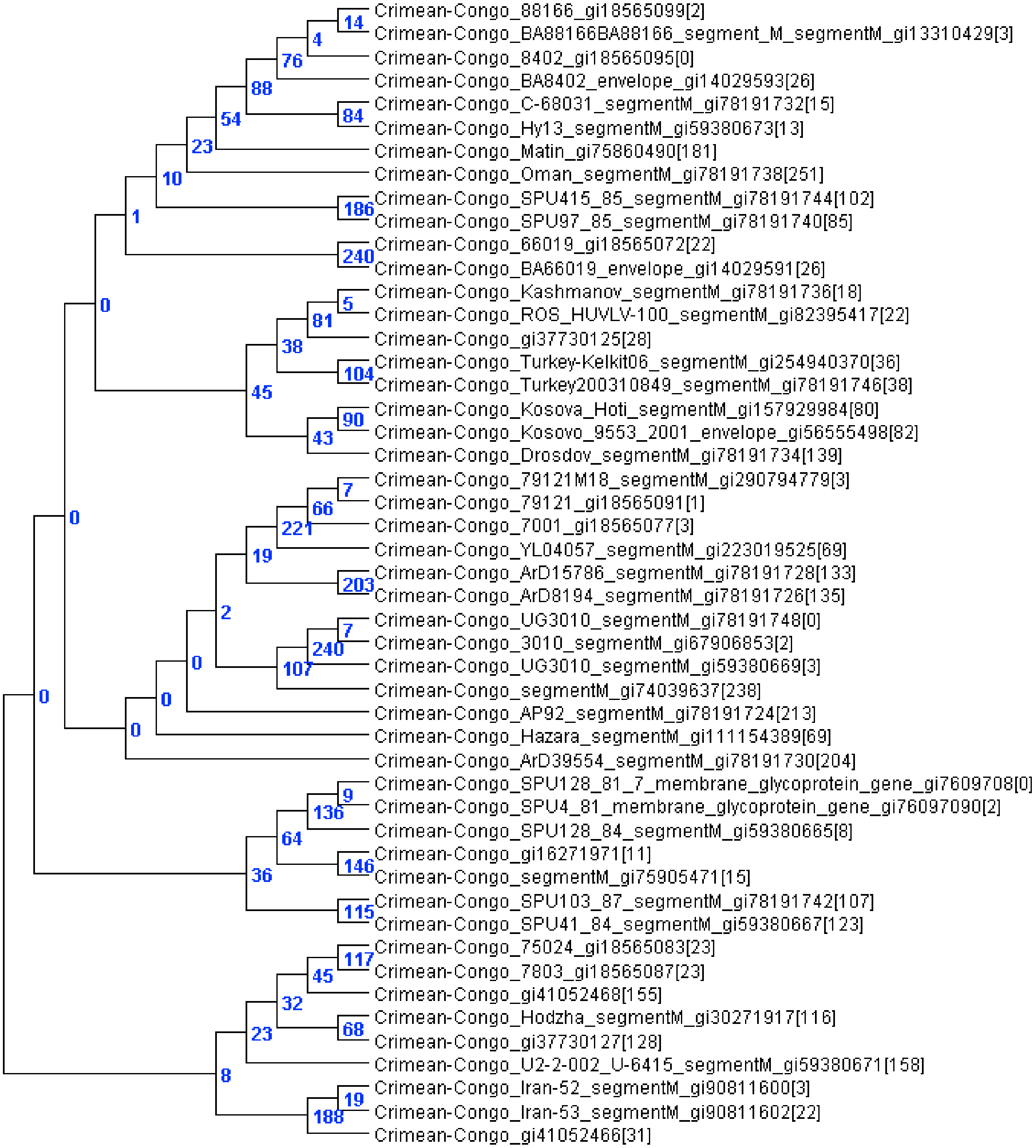
Crimean-Congo_Semunya_L_gi71648470[373]

CCHF M segment

Number_SNPs: 4720
Number_Homoplastic_SNPs from MSA tree: 680
Number_Homoplastic_SNPs from SNP Hamming distance tree (shown below): 585
Number_Homoplastic_SNPs from SNP RAxML tree: 791
All strains can be uniquely resolved on the basis of SNPs.
Number of SNP microarray probes:
144,741 probes for all alleles
38,729 probes for observed alleles only



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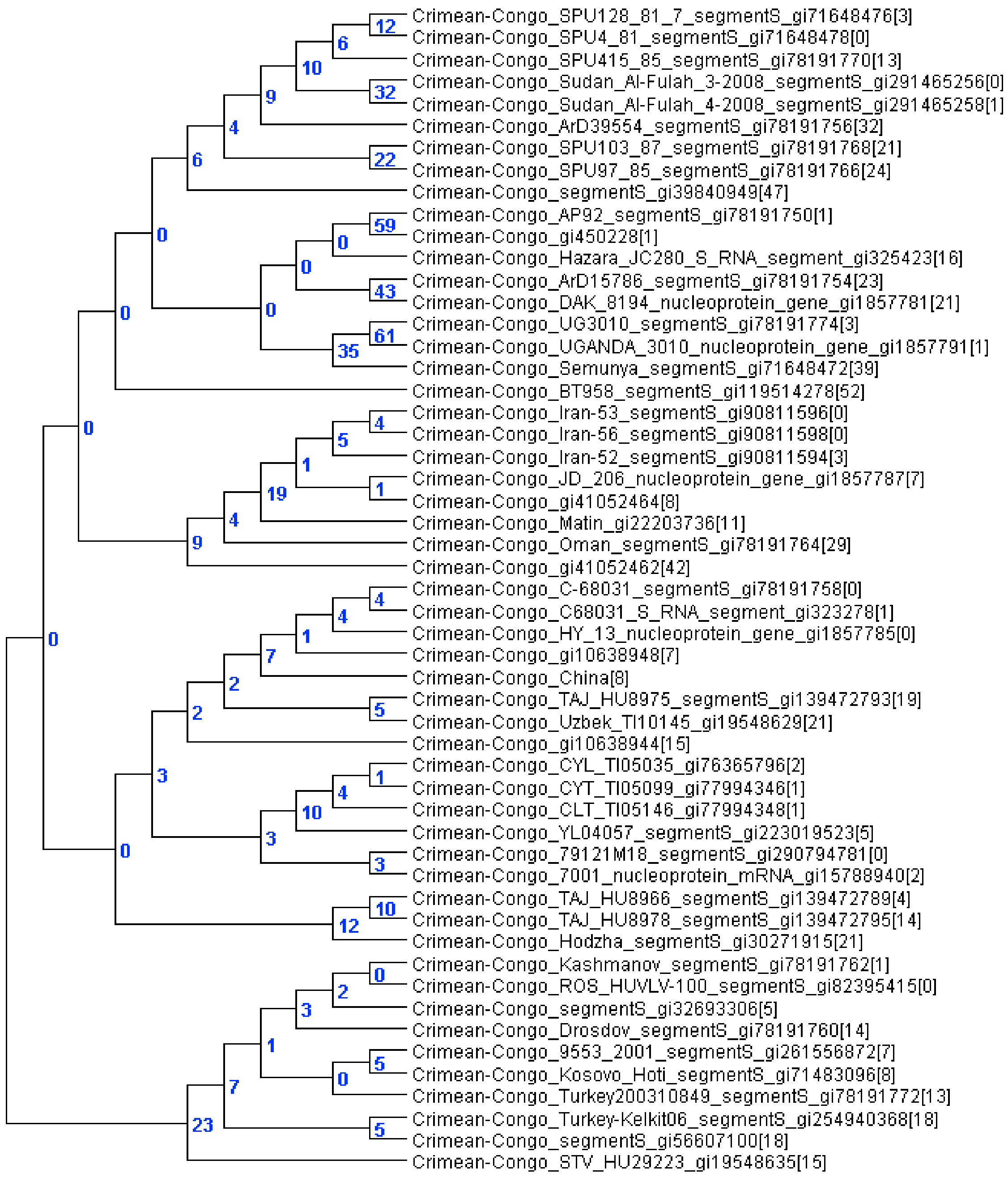
CCHF S segment

Number_SNPs: 1073
Number_Homoplastic_SNPs from MSA tree: 294
Number_Homoplastic_SNPs from SNP Hamming distance tree (shown below): 266
Number_Homoplastic_SNPs from SNP RAxML tree: 326
All strains can be uniquely resolved on the basis of SNPs.
Number of SNP microarray probes:
40,437 probes for all alleles
11,145 probes for observed alleles only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Crimean-Congo_gi10638944[15]
Crimean-Congo_CYL_TI05035_gi76365796[2]
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Crimean-Congo_CYT_TI05099_gi77994346[1]
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Crimean-Congo_CLT_TI05146_gi77994348[1]
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Crimean-Congo_YL04057_segmentS_gi223019523[5]
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Crimean-Congo_Hodzha_segmentS_gi30271915[21]
Crimean-Congo_Kashmanov_segmentS_gi78191762[1]
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Crimean-Congo_Drosdov_segmentS_gi78191760[14]
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Crimean-Congo_Kosovo_Hoti_segmentS_gi71483096[8]
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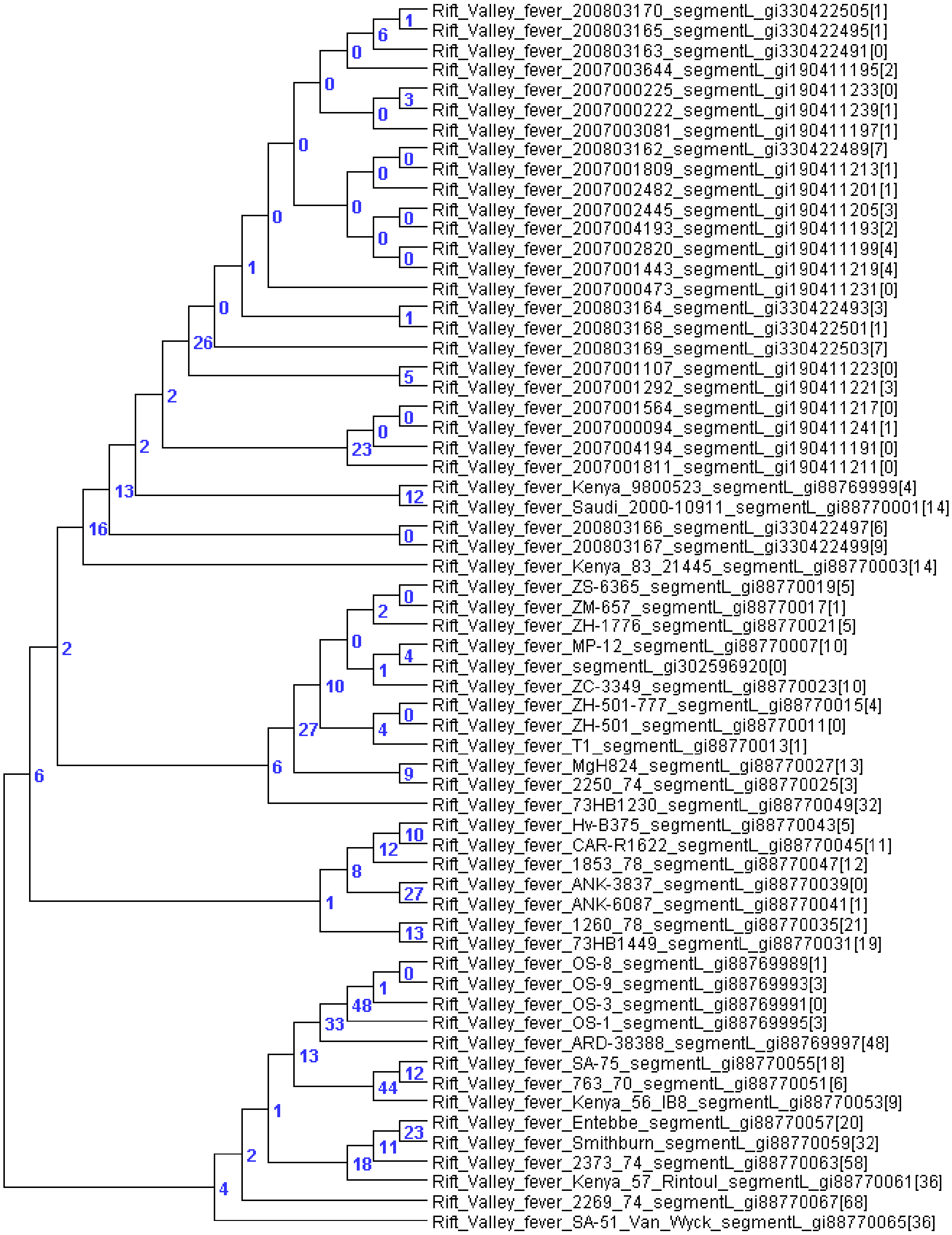
Crimean-Congo_Turkey-Kelkit06_segmentS_gi254940368[18]
23
5
Crimean-Congo_segmentS_gi56607100[18]
Crimean-Congo_STV_HU29223_gi19548635[15]

RVF L segment

Number_SNPs: 1190
Number_Homoplastic_SNPs from MSA tree (shown below): 271
Number_Homoplastic_SNPs from SNP Hamming distance tree: 276
Number_Homoplastic_SNPs from SNP RAxML tree: 271
All strains can be uniquely resolved on the basis of SNPs.
Number of SNP microarray probes:
39,213 probes for all alleles
12,181 probes for observed alleles only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Rift_Valley_fever_2007000225_segmentL_gi190411233[0]
3
Rift_Valley_fever_2007000222_segmentL_gi190411239[1]
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Rift_Valley_fever_2007003081_segmentL_gi190411197[1]
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Rift_Valley_fever_200803162_segmentL_gi330422489[7]
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Rift_Valley_fever_2007001809_segmentL_gi190411213[1]
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Rift_Valley_fever_2007001443_segmentL_gi190411219[4]
Rift_Valley_fever_2007000473_segmentL_gi190411231[0]
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Rift_Valley_fever_200803164_segmentL_gi330422493[3]
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2
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Rift_Valley_fever_2007001811_segmentL_gi190411211[0]
Rift_Valley_fever_Kenya_9800523_segmentL_gi88769999[4]
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12
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6
9
6
Rift_Valley_fever_2250_74_segmentL_gi88770025[3]
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Rift_Valley_fever_Kenya_57_Rintoul_segmentL_gi88770061[36]
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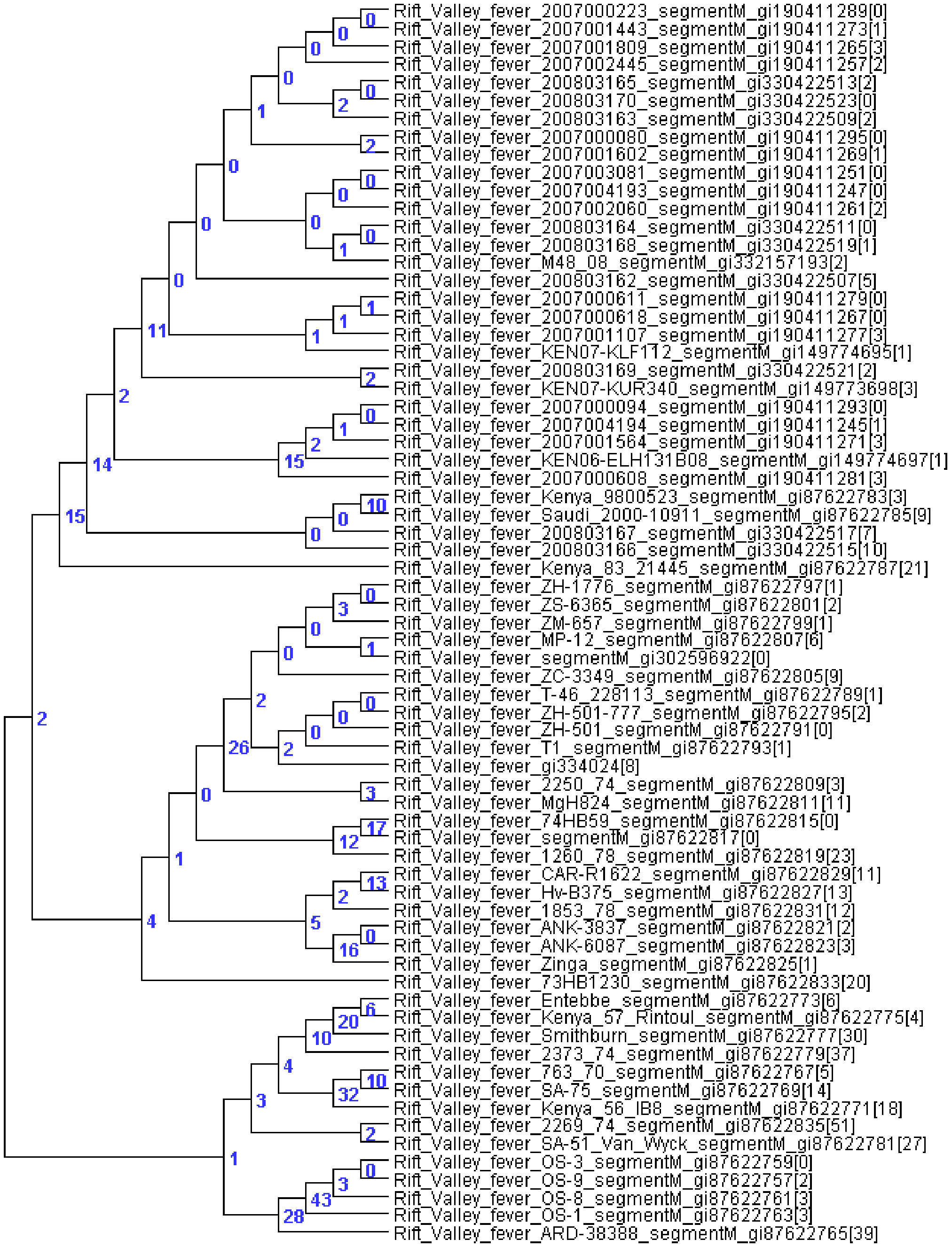
Rift_Valley_fever_SA-51_Van_Wyck_segmentL_gi88770065[36]

RVF M segment

Number_SNPs: 897
Number_Homoplastic_SNPs from MSA tree: 192
Number_Homoplastic_SNPs from SNP Hamming distance tree (shown below): 189
Number_Homoplastic_SNPs from SNP RAxML tree: 191
All strains can be uniquely resolved on the basis of SNPs.
Number of SNP microarray probes:
33,025 probes for all alleles
9,888 probes for observed alleles only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Rift_Valley_fever_200803163_segmentM_gi330422509[2]
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Rift_Valley_fever_2007004193_segmentM_gi190411247[0]
Rift_Valley_fever_2007002060_segmentM_gi190411261[2]
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Rift_Valley_fever_200803168_segmentM_gi330422519[1]
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Rift_Valley_fever_Saudi_2000-10911_segmentM_gi87622785[9]
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Rift_Valley_fever_ANK-6087_segmentM_gi87622823[3]
Rift_Valley_fever_Zinga_segmentM_gi87622825[1]
Rift_Valley_fever_73HB1230_segmentM_gi87622833[20]
Rift_Valley_fever_Entebbe_segmentM_gi87622773[6]
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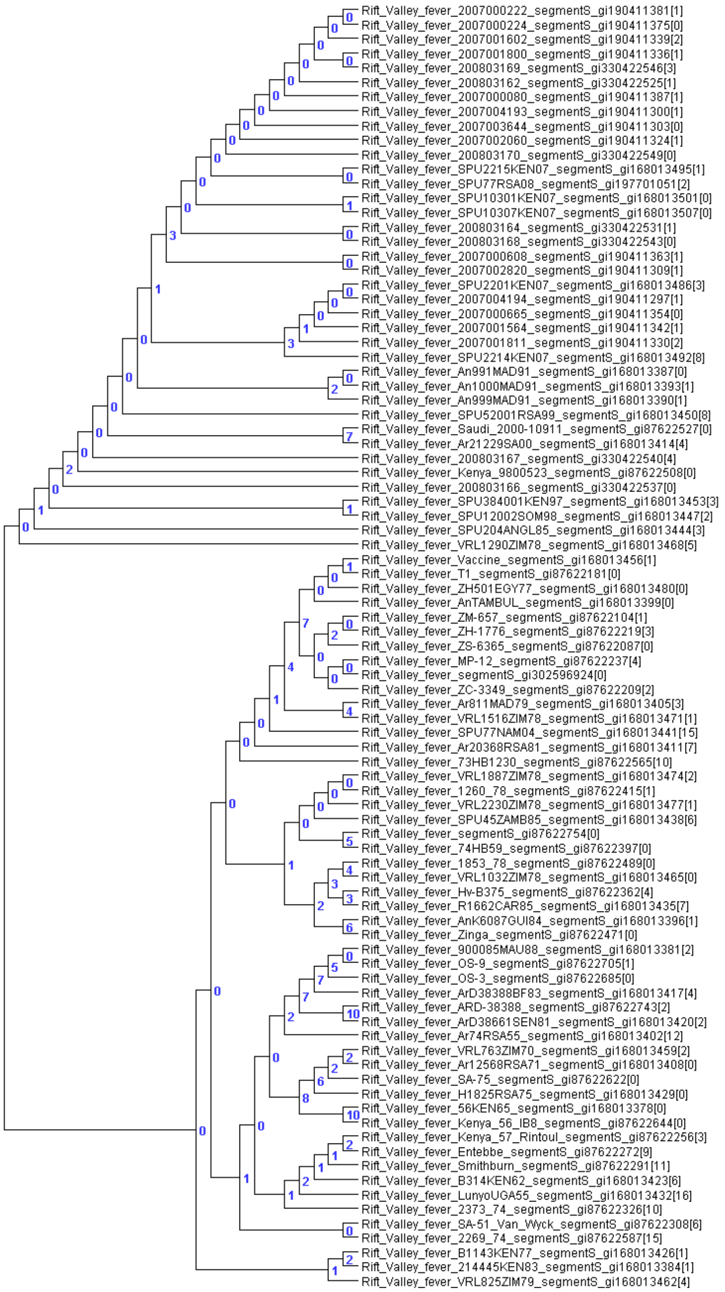
Rift_Valley_fever_OS-1_segmentM_gi87622763[3]
Rift_Valley_fever_ARD-38388_segmentM_gi87622765[39]

RVF S segment

Number_SNPs: 436
Number_Homoplastic_SNPs from MSA tree: 107
Number_Homoplastic_SNPs from SNP Hamming distance tree: 110
Number_Homoplastic_SNPs from SNP RAxML tree (shown below): 106
Only 2 strains cannot be uniquely resolved on the basis of SNPs:
Rift_Valley_fever_200803166_segmentS_gi330422537
Rift_Valley_fever_Kenya_9800523_segmentS_gi87622508
Number of SNP microarray probes:
17,993 probes for all alleles
5,381 probes for observed alleles only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Rift_Valley_fever_SPU77RSA08_segmentS_gi197701051[2]
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Rift_Valley_fever_SPU10307KEN07_segmentS_gi168013507[0]
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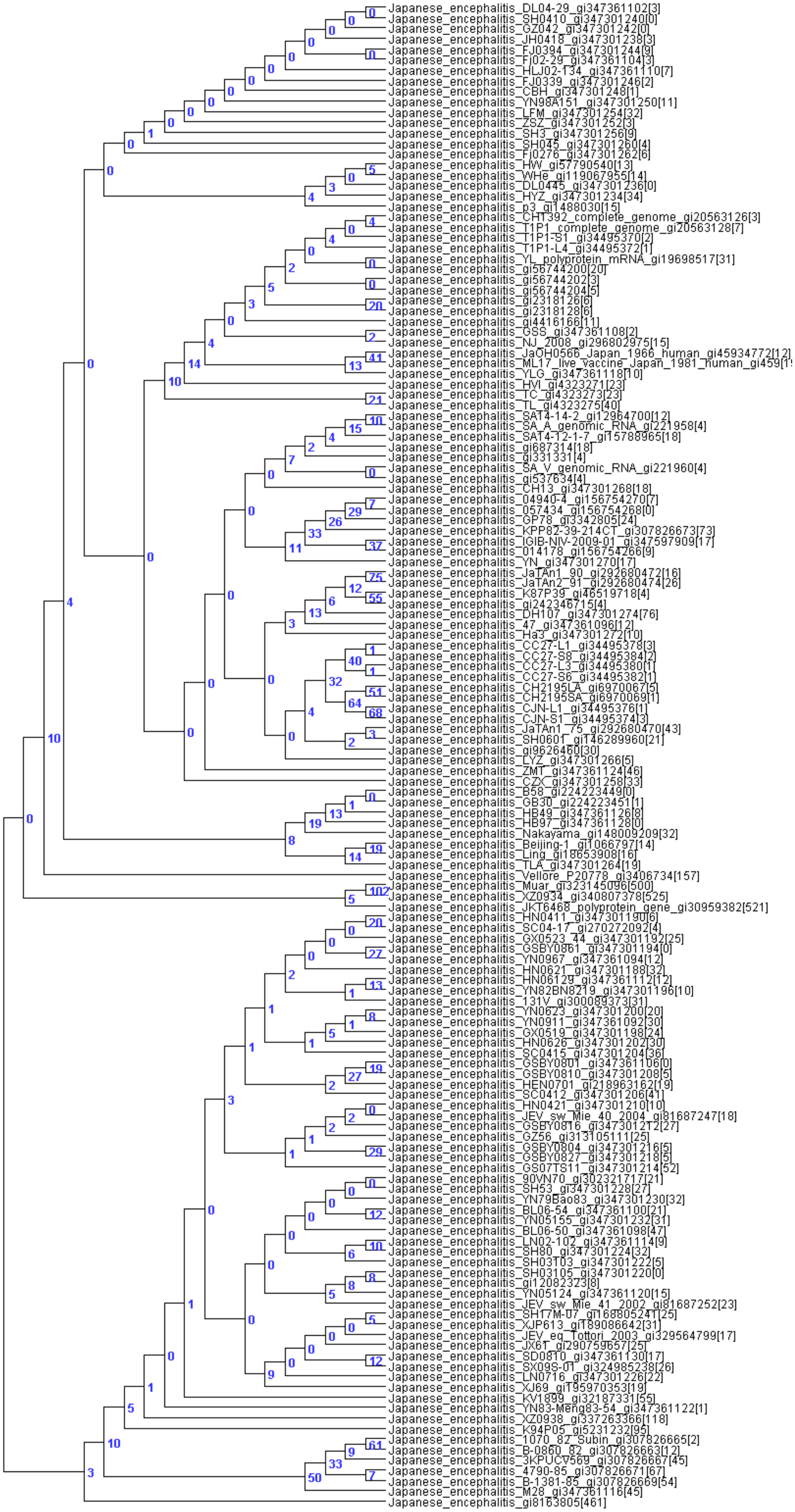
JEV

Number_SNPs: 6759
Number_Homoplastic_SNPs from MSA tree: 2050
Number_Homoplastic_SNPs from SNP Hamming distance tree (shown below): 2018
Number_Homoplastic_SNPs from SNP RAxML tree: 2139
All strains can be uniquely resolved on the basis of SNPs.
Number of SNP microarray probes:
273,205 probes for all alleles
78,468 probes for observed alleles only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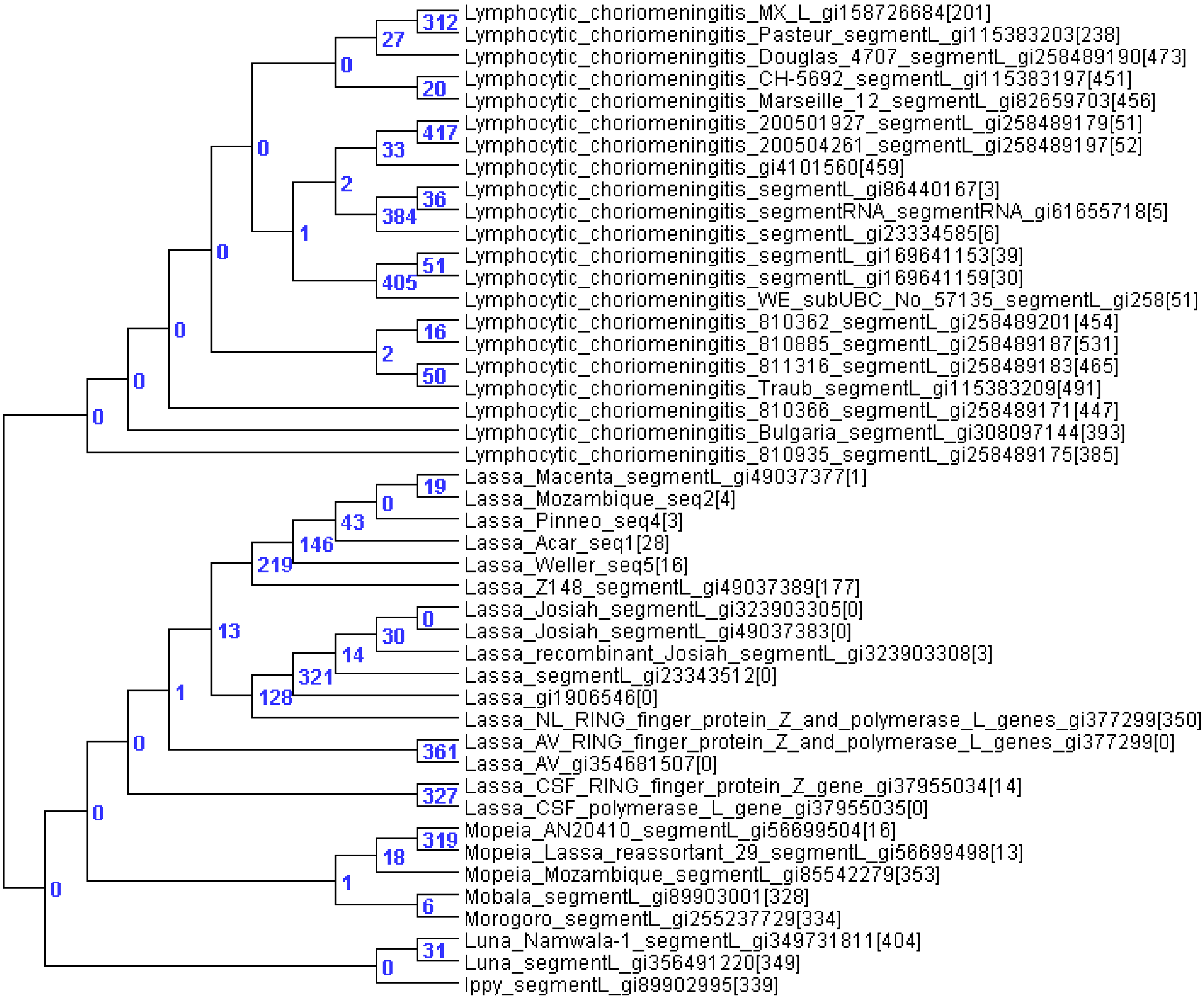
Japanese_encephalitis_B-1381-85_gi307826669[54]
Japanese_encephalitis_M28_gi347361116[45]
Japanese_encephalitis_gi8163805[461]

Old World Arenaviruses L segment

Number_SNPs: 7556
Number_Homoplastic_SNPs from MSA tree: 698
Number_Homoplastic_SNPs from SNP Hamming distance tree (shown below): 417
Number_Homoplastic_SNPs from SNP RAxML tree: 606
All strains can be uniquely resolved on the basis of SNPs.
Number of SNP microarray probes:
173,157 probes for all alleles
44,031 probes for observed alleles only



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Lymphocytic_choriomeningitis_CH-5692_segmentL_gi115383197[451]
Lymphocytic_choriomeningitis_Marseille_12_segmentL_gi82659703[456]
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Lymphocytic_choriomeningitis_200501927_segmentL_gi258489179[51]
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Lymphocytic_choriomeningitis_200504261_segmentL_gi258489197[52]
Lymphocytic_choriomeningitis_gi4101560[459]
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36
Lymphocytic_choriomeningitis_segmentL_gi86440167[3]
384
Lymphocytic_choriomeningitis_segmentRNA_segmentRNA_gi61655718[5]
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Lymphocytic_choriomeningitis_segmentL_gi23334585[6]
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51
Lymphocytic_choriomeningitis_segmentL_gi169641153[39]
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Lymphocytic_choriomeningitis_segmentL_gi169641159[30]
Lymphocytic_choriomeningitis_WE_subUBC_No_57135_segmentL_gi258[51]
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Lymphocytic_choriomeningitis_810362_segmentL_gi258489201[454]
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Lymphocytic_choriomeningitis_810885_segmentL_gi258489187[531]
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Lymphocytic_choriomeningitis_Traub_segmentL_gi115383209[491]
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Lassa_Pinneo_seq4[3]
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Lassa_Acar_seq1[28]
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Lassa_Weller_seq5[16]
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Lassa_Josiah_segmentL_gi323903305[0]
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Lassa_Josiah_segmentL_gi49037383[0]
14
Lassa_recombinant_Josiah_segmentL_gi323903308[3]
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Lassa_segmentL_gi23343512[0]
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Lassa_gi1906546[0]
Lassa_NL_RING_finger_protein_Z_and_polymerase_L_genes_gi377299[350]
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Lassa_AV_RING_finger_protein_Z_and_polymerase_L_genes_gi377299[0]
Lassa_AV_gi354681507[0]
327
Lassa_CSF_RING_finger_protein_Z_gene_gi37955034[14]
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Lassa_CSF_polymerase_L_gene_gi37955035[0]
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Mopeia_AN20410_segmentL_gi56699504[16]
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Mopeia_Lassa_reassortant_29_segmentL_gi56699498[13]
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Mopeia_Mozambique_segmentL_gi85542279[353]
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6
Mobala_segmentL_gi89903001[328]
Morogoro_segmentL_gi255237729[334]
31
Luna_Namwala-1_segmentL_gi349731811[404]
0
Luna_segmentL_gi356491220[349]
Ippy_segmentL_gi89902995[339]

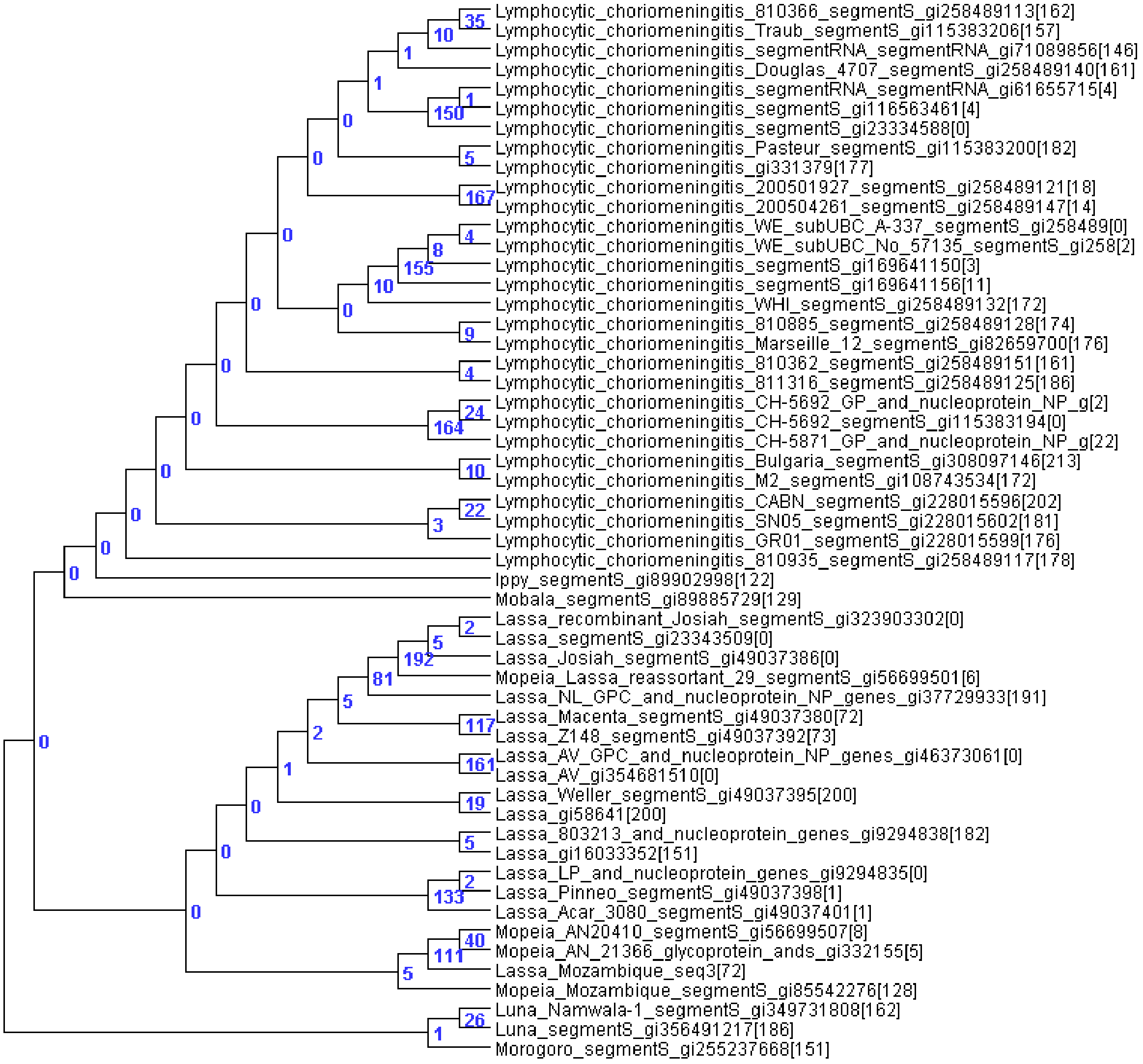
Old World Arenaviruses S segment

Number_SNPs: 4657
Number_Homoplastic_SNPs from MSA tree: 602
Number_Homoplastic_SNPs from SNP Hamming distance tree (shown below): 484
Number_Homoplastic_SNPs from SNP RAxML tree: 576
There are 2 pairs of strains that cannot be uniquely resolved on the basis of SNPs:

1 Lassa_AV_GPC_and_nucleoprotein_NP_genes_gi46373061
1 Lassa_AV_gi354681510

2 Lassa_recombinant_Josiah_segmentS_gi323903302
2 Lassa_segmentS_gi23343509

Number of SNP microarray probes:
120,873 probes for all alleles
30,698 probes for observed alleles only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Lymphocytic_choriomeningitis_Traub_segmentS_gi115383206[157]
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Lymphocytic_choriomeningitis_Douglas_4707_segmentS_gi258489140[161]
Lymphocytic_choriomeningitis_segmentRNA_segmentRNA_gi61655715[4]
Lymphocytic_choriomeningitis_segmentS_gi116563461[4]
Lymphocytic_choriomeningitis_segmentS_gi23334588[0]
Lymphocytic_choriomeningitis_Pasteur_segmentS_gi115383200[182]
Lymphocytic_choriomeningitis_gi331379[177]
Lymphocytic_choriomeningitis_200501927_segmentS_gi258489121[18]
Lymphocytic_choriomeningitis_200504261_segmentS_gi258489147[14]
Lymphocytic_choriomeningitis_WE_subUBC_A-337_segmentS_gi258489[0]
Lymphocytic_choriomeningitis_WE_subUBC_No_57135_segmentS_gi258[2]
Lymphocytic_choriomeningitis_segmentS_gi169641150[3]
Lymphocytic_choriomeningitis_segmentS_gi169641156[11]
Lymphocytic_choriomeningitis_WHI_segmentS_gi258489132[172]
Lymphocytic_choriomeningitis_810885_segmentS_gi258489128[174]
Lymphocytic_choriomeningitis_Marseille_12_segmentS_gi82659700[176]
Lymphocytic_choriomeningitis_810362_segmentS_gi258489151[161]
Lymphocytic_choriomeningitis_811316_segmentS_gi258489125[186]
Lymphocytic_choriomeningitis_CH-5692_GP_and_nucleoprotein_NP_g[2]
Lymphocytic_choriomeningitis_CH-5692_segmentS_gi115383194[0]
Lymphocytic_choriomeningitis_CH-5871_GP_and_nucleoprotein_NP_g[22]
Lymphocytic_choriomeningitis_Bulgaria_segmentS_gi308097146[213]
Lymphocytic_choriomeningitis_M2_segmentS_gi108743534[172]
Lymphocytic_choriomeningitis_CABN_segmentS_gi228015596[202]
Lymphocytic_choriomeningitis_SN05_segmentS_gi228015602[181]
Lymphocytic_choriomeningitis_GR01_segmentS_gi228015599[176]
Lymphocytic_choriomeningitis_810935_segmentS_gi258489117[178]
Ippy_segmentS_gi89902998[122]
Mobala_segmentS_gi89885729[129]
Lassa_recombinant_Josiah_segmentS_gi323903302[0]
Lassa_segmentS_gi23343509[0]
Lassa_Josiah_segmentS_gi49037386[0]
Mopeia_Lassa_reassortant_29_segmentS_gi56699501[6]
Lassa_NL_GPC_and_nucleoprotein_NP_genes_gi37729933[191]
Lassa_Macenta_segmentS_gi49037380[72]
Lassa_Z148_segmentS_gi49037392[73]
Lassa_AV_GPC_and_nucleoprotein_NP_genes_gi46373061[0]
Lassa_AV_gi354681510[0]
Lassa_Weller_segmentS_gi49037395[200]
Lassa_gi58641[200]
Lassa_803213_and_nucleoprotein_genes_gi9294838[182]
Lassa_gi16033352[151]
Lassa_LP_and_nucleoprotein_genes_gi9294835[0]
Lassa_Pinneo_segmentS_gi49037398[1]
Lassa_Acar_3080_segmentS_gi49037401[1]
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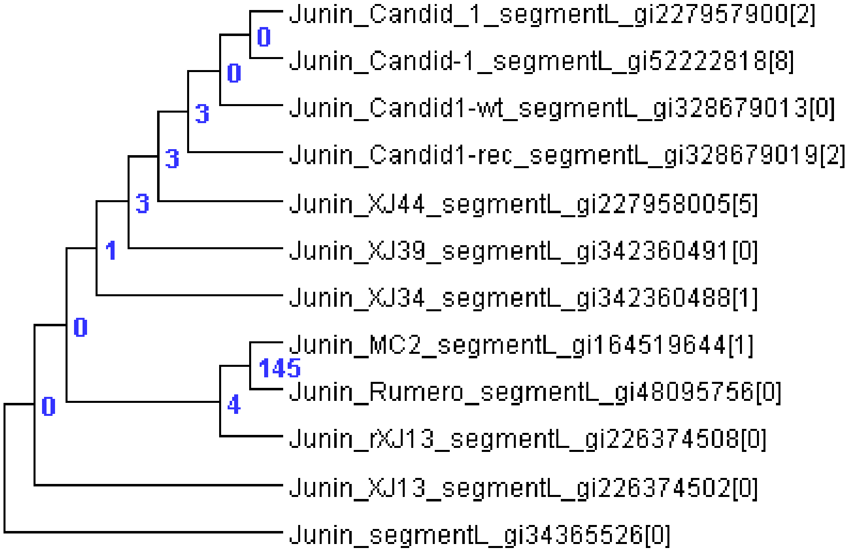
Mopeia_AN_21366_glycoprotein_ands_gi332155[5]
Lassa_Mozambique_seq3[72]
Mopeia_Mozambique_segmentS_gi85542276[128]
Luna_Namwala-1_segmentS_gi349731808[162]
Luna_segmentS_gi356491217[186]
Morogoro_segmentS_gi255237668[151]

Junin L segment

Number_SNPs: 182
Number_Homoplastic_SNPs from MSA tree: 7
Number_Homoplastic_SNPs from SNP Hamming distance tree: 5
Number_Homoplastic_SNPs from SNP RAxML tree (shown below): 4
All strains can be uniquely resolved on the basis of SNPs.
Number of SNP microarray probes:
2397 probes for all alleles
812 probes for observed alleles only


Junin_Candid_1_segmentL_gi227957900[2]
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Junin_Candid1-wt_segmentL_gi328679013[0]
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Junin_Candid1-rec_segmentL_gi328679019[2]
3
Junin_XJ44_segmentL_gi227958005[5]
3
Junin_XJ39_segmentL_gi342360491[0]
1
Junin_XJ34_segmentL_gi342360488[1]
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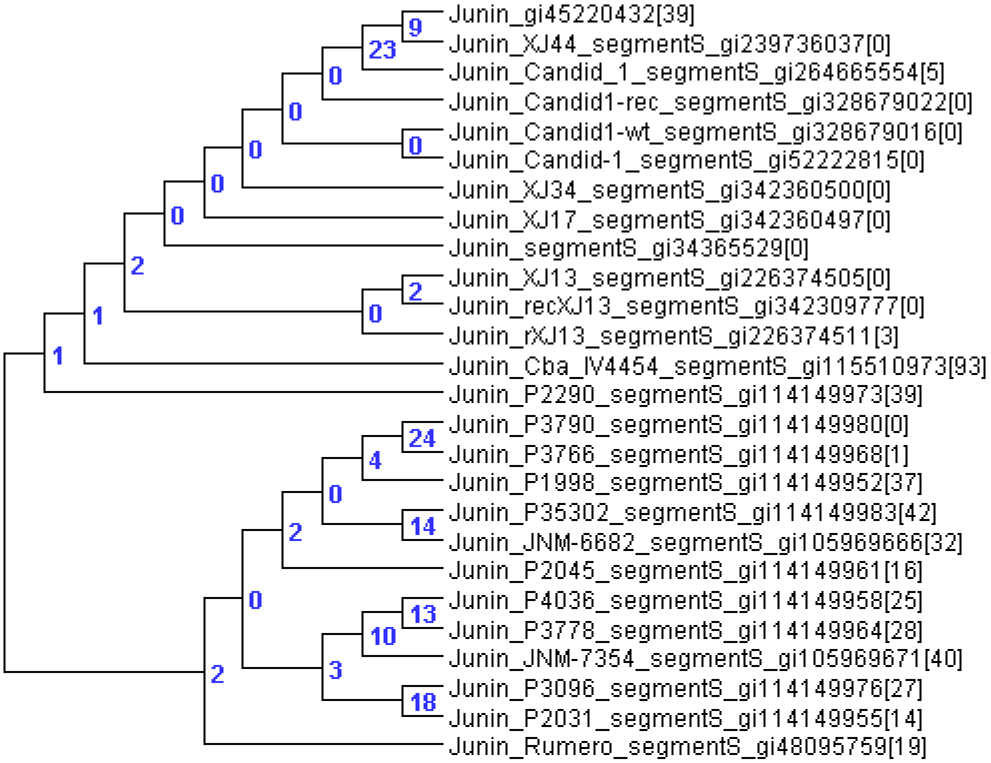
Junin_MC2_segmentL_gi164519644[1]
145
Junin_Rumero_segmentL_gi48095756[0]
0
4
Junin_rXJ13_segmentL_gi226374508[0]
Junin_XJ13_segmentL_gi226374502[0]
Junin_segmentL_gi34365526[0]

Junin S segment

Number_SNPs: 660
Number_Homoplastic_SNPs from MSA tree: 162
Number_Homoplastic_SNPs from SNP Hamming distance tree: 161
Number_Homoplastic_SNPs from SNP RAxML tree (shown below): 160
All strains can be uniquely resolved on the basis of SNPs.
Number of SNP microarray probes:
20,341 probes for all alleles
6,100 probes for observed alleles only



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Junin_Candid-1_segmentS_gi52222815[0]
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Junin_segmentS_gi34365529[0]
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Junin_XJ13_segmentS_gi226374505[0]
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Junin_recXJ13_segmentS_gi342309777[0]
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Junin_rXJ13_segmentS_gi226374511[3]
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Junin_Cba_IV4454_segmentS_gi115510973[93]
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Junin_P2290_segmentS_gi114149973[39]
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Junin_P3766_segmentS_gi114149968[1]
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Junin_P1998_segmentS_gi114149952[37]
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14
Junin_JNM-6682_segmentS_gi105969666[32]
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Junin_P2045_segmentS_gi114149961[16]
Junin_P4036_segmentS_gi114149958[25]
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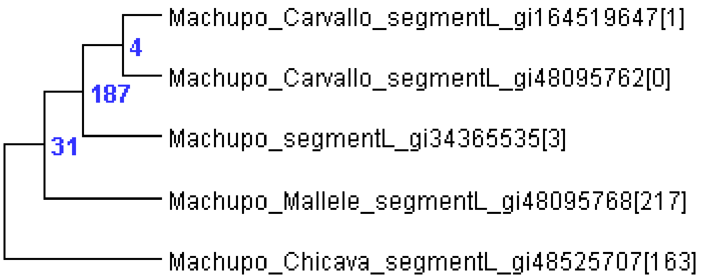
13
Junin_P3778_segmentS_gi114149964[28]
10
Junin_JNM-7354_segmentS_gi105969671[40]
3
Junin_P3096_segmentS_gi114149976[27]
2
18
Junin_P2031_segmentS_gi114149955[14]
Junin_Rumero_segmentS_gi48095759[19]

Machupo L segment

Number_SNPs: 373
Number_Homoplastic_SNPs from MSA tree (shown below): 0
Number_Homoplastic_SNPs from SNP Hamming distance tree: 0
Number_Homoplastic_SNPs from SNP RAxML tree: 0
All strains can be uniquely resolved on the basis of SNPs.
Number of SNP microarray probes:
6173 probes for all alleles
1719 probes for observed alleles only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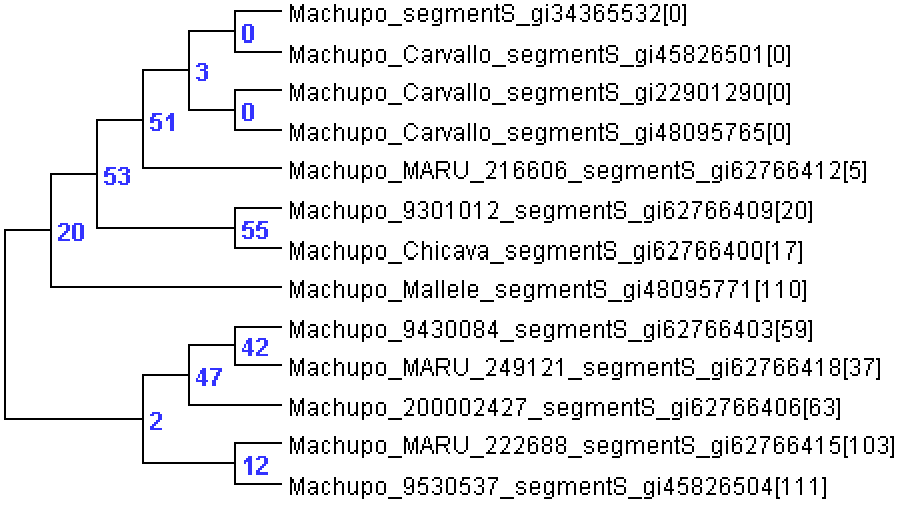
Machupo_Carvallo_segmentL_gi48095762[0]
187
Machupo_segmentL_gi34365535[3]
31
Machupo_Mallele_segmentL_gi48095768[217]
Machupo_Chicava_segmentL_gi48525707[163]

Machupo S segment

Number_SNPs: 614
Number_Homoplastic_SNPs from MSA tree (shown below): 54
Number_Homoplastic_SNPs from SNP Hamming distance tree: 54
Number_Homoplastic_SNPs from SNP RAxML tree: 56
The following 4 strains cannot be uniquely resolved on the basis of SNPs:

1	Machupo_Carvallo_segmentS_gi22901290
1	Machupo_Carvallo_segmentS_gi45826501
1	Machupo_Carvallo_segmentS_gi48095765
1	Machupo_segmentS_gi34365532

Number of SNP microarray probes:
15,309 probes for all alleles
4,336 probes for observed alleles only


Machupo_segmentS_gi34365532[0]
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Machupo_Carvallo_segmentS_gi22901290[0]
51
0
Machupo_Carvallo_segmentS_gi48095765[0]
53
Machupo_MARU_216606_segmentS_gi62766412[5]
Machupo_9301012_segmentS_gi62766409[20]
20
55
Machupo_Chicava_segmentS_gi62766400[17]
Machupo_Mallele_segmentS_gi48095771[110]
Machupo_9430084_segmentS_gi62766403[59]
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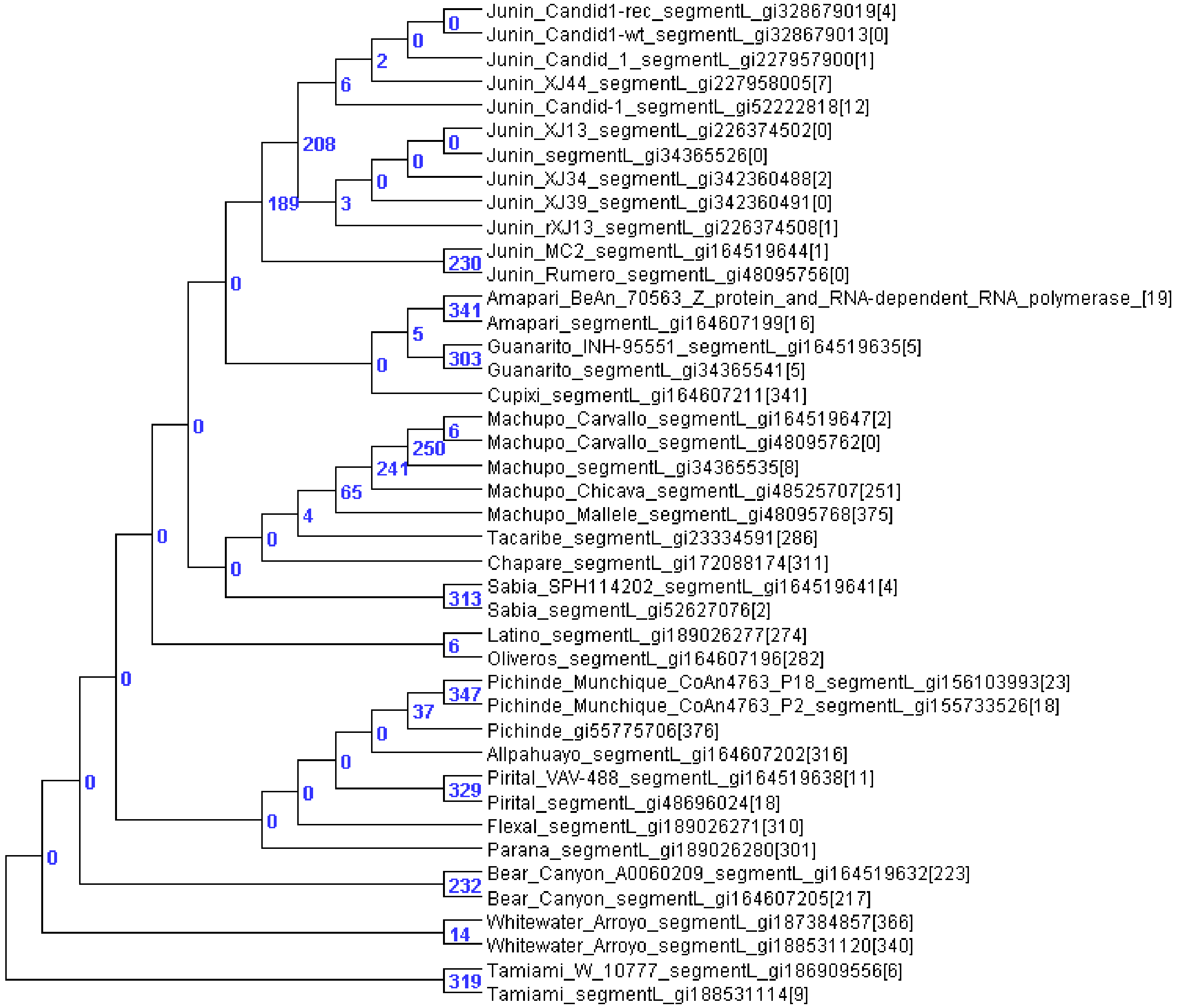
47
Machupo_MARU_249121_segmentS_gi62766418[37]
Machupo_200002427_segmentS_gi62766406[63]
2
Machupo_MARU_222688_segmentS_gi62766415[103]
12
Machupo_9530537_segmentS_gi45826504[111]

New World Arenaviruses L segment (including Machupo, Junin, and others)

Number_SNPs: 4389
Number_Homoplastic_SNPs from MSA tree: 79
Number_Homoplastic_SNPs from SNP Hamming distance tree (shown below): 50
Number_Homoplastic_SNPs from SNP RAxML tree: 233
All strains can be uniquely resolved on the basis of SNPs.
Number of SNP microarray probes:
80,973 probes for all alleles
20,800 probes for observed alleles only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Junin_XJ44_segmentL_gi227958005[7]
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Junin_XJ34_segmentL_gi342360488[2]
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Junin_MC2_segmentL_gi164519644[1]
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Junin_Rumero_segmentL_gi48095756[0]
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Guanarito_INH-95551_segmentL_gi164519635[5]
303
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Cupixi_segmentL_gi164607211[341]
Machupo_Carvallo_segmentL_gi164519647[2]
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Machupo_Carvallo_segmentL_gi48095762[0]
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Machupo_segmentL_gi34365535[8]
241
Machupo_Chicava_segmentL_gi48525707[251]
65
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Chapare_segmentL_gi172088174[311]
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Sabia_SPH114202_segmentL_gi164519641[4]
313
Sabia_segmentL_gi52627076[2]
Latino_segmentL_gi189026277[274]
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Oliveros_segmentL_gi164607196[282]
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Pichinde_Munchique_CoAn4763_P18_segmentL_gi156103993[23]
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Pichinde_Munchique_CoAn4763_P2_segmentL_gi155733526[18]
Pichinde_gi55775706[376]
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Allpahuayo_segmentL_gi164607202[316]
Pirital_VAV-488_segmentL_gi164519638[11]
0
329
0
Pirital_segmentL_gi48696024[18]
0
Flexal_segmentL_gi189026271[310]
Parana_segmentL_gi189026280[301]
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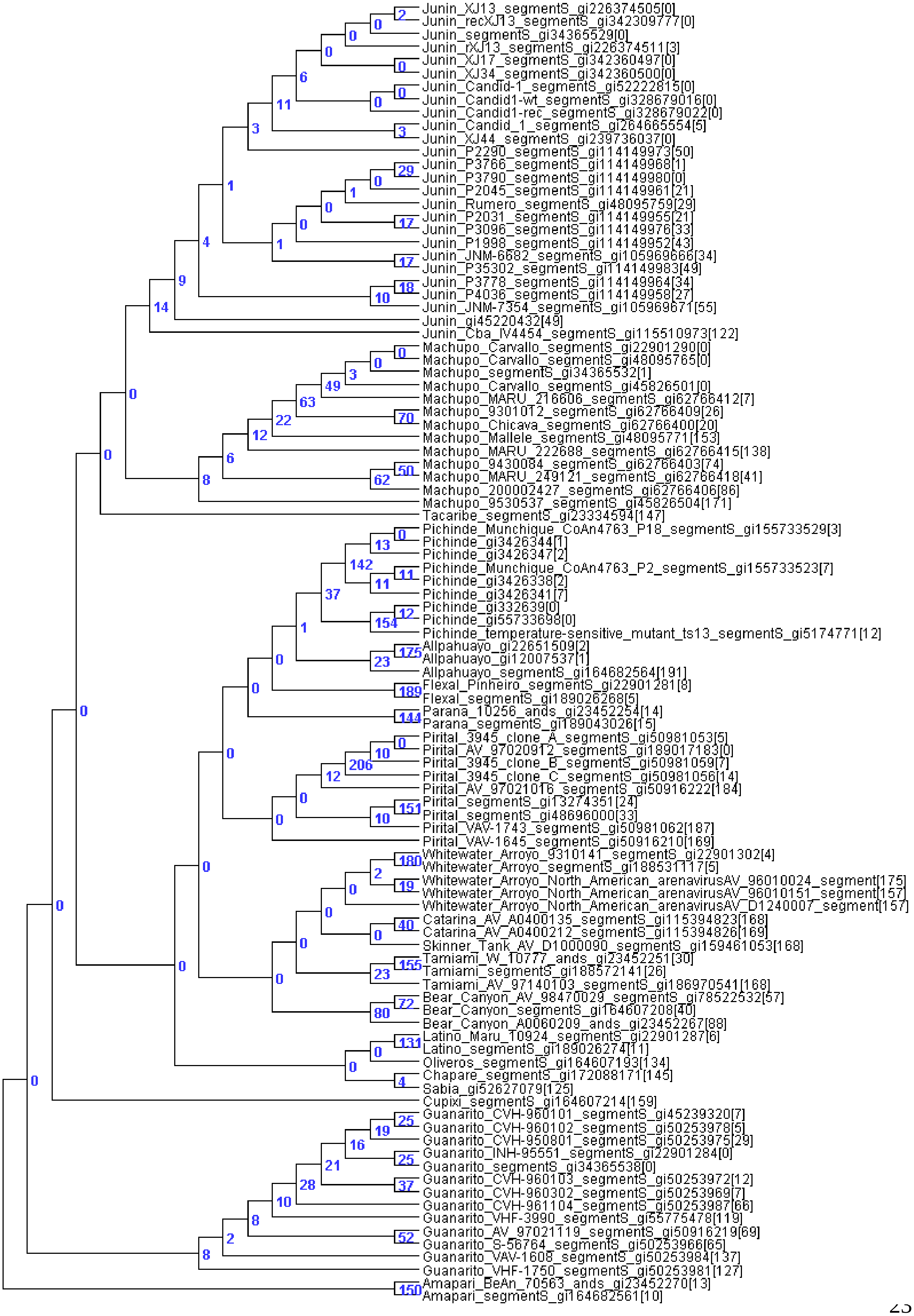
Bear_Canyon_A0060209_segmentL_gi164519632[223]
232
Bear_Canyon_segmentL_gi164607205[217]
Whitewater_Arroyo_segmentL_gi187384857[366]
14
Whitewater_Arroyo_segmentL_gi188531120[340]
Tamiami_W_10777_segmentL_gi186909556[6]
319
Tamiami_segmentL_gi188531114[9]

New World Arenaviruses S segment (including Machupo, Junin, and others)

Number_SNPs: 5410
Number_Homoplastic_SNPs from MSA tree: 654
Number_Homoplastic_SNPs from SNP Hamming distance tree (shown below): 521
Number_Homoplastic_SNPs from SNP RAxML tree: 724
The following 2 pairs of strains cannot be uniquely resolved on the basis of SNP:

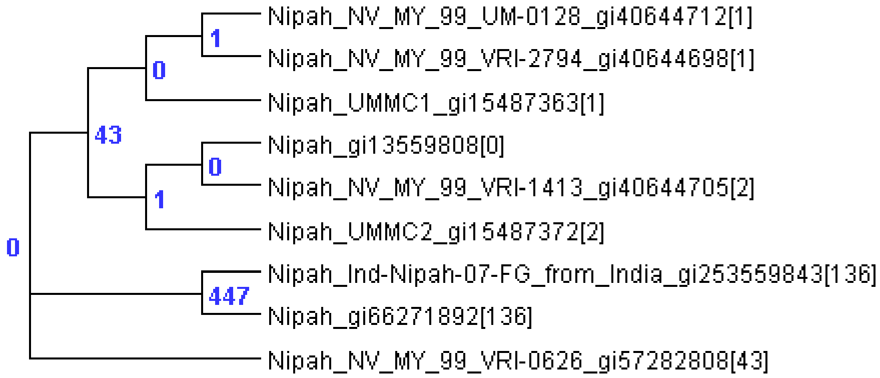
1	Machupo_Carvallo_segmentS_gi22901290
1	Machupo_Carvallo_segmentS_gi48095765
2	Pichinde_gi332639
2	Pichinde_gi55733698

Number of SNP microarray probes:
139,505 probes for all alleles
37,249 probes for observed alleles only


Junin_XJ13_segmentS_gi226374505[0]
Junin_recXJ13_segmentS_gi342309777[0]
Junin_segmentS_gi34365529[0]
Junin_rXJ13_segmentS_gi226374511[3]
Junin_XJ17_segmentS_gi342360497[0]
Junin_XJ34_segmentS_gi342360500[0]
Junin_Candid-1_segmentS_gi52222815[0]
Junin_Candid1-wt_segmentS_gi328679016[0]
Junin_Candid1-rec_segmentS_gi328679022[0]
Junin_Candid_1_segmentS_gi264665554[5]
Junin_XJ44_segmentS_gi239736037[0]
Junin_P2290_segmentS_gi114149973[50]
Junin_P3766_segmentS_gi114149968[1]
Junin_P3790_segmentS_gi114149980[0]
Junin_P2045_segmentS_gi114149961[21]
Junin_Rumero_segmentS_gi48095759[29]
Junin_P2031_segmentS_gi114149955[21]
Junin_P3096_segmentS_gi114149976[33]
Junin_P1998_segmentS_gi114149952[43]
Junin_JNM-6682_segmentS_gi105969666[34]
Junin_P35302_segmentS_gi114149983[49]
Junin_P3778_segmentS_gi114149964[34]
Junin_P4036_segmentS_gi114149958[27]
Junin_JNM-7354_segmentS_gi105969671[55]
Junin_gi45220432[49]
Junin_Cba_IV4454_segmentS_gi115510973[122]
Machupo_Carvallo_segmentS_gi22901290[0]
Machupo_Carvallo_segmentS_gi48095765[0]
Machupo_segmentS_gi34365532[1]
Machupo_Carvallo_segmentS_gi45826501[0]
Machupo_MARU_216606_segmentS_gi62766412[7]
Machupo_9301012_segmentS_gi62766409[26]
Machupo_Chicava_segmentS_gi62766400[20]
Machupo_Mallele_segmentS_gi48095771[153]
Machupo_MARU_222688_segmentS_gi62766415[138]
Machupo_9430084_segmentS_gi62766403[74]
Machupo_MARU_249121_segmentS_gi62766418[41]
Machupo_200002427_segmentS_gi62766406[86]
Machupo_9530537_segmentS_gi45826504[171]
Tacaribe_segmentS_gi23334594[147]
Pichinde_Munchique_CoAn4763_P18_segmentS_gi155733529[3]
Pichinde_gi3426344[1]
Pichinde_gi3426347[2]
Pichinde_Munchique_CoAn4763_P2_segmentS_gi155733523[7]
Pichinde_gi3426338[2]
Pichinde_gi3426341[7]
Pichinde_gi332639[0]
Pichinde_gi55733698[0]
Pichinde_temperature-sensitive_mutant_ts13_segmentS_gi5174771[12]
Allpahuayo_gi22651509[2]
Allpahuayo_gi12007537[1]
Allpahuayo_segmentS_gi164682564[191]
Flexal_Pinheiro_segmentS_gi22901281[8]
Flexal_segmentS_gi189026268[5]
Parana_10256_ands_gi23452254[14]
Parana_segmentS_gi189043026[15]
Pirital_3945_clone_A_segmentS_gi50981053[5]
Pirital_AV_97020912_segmentS_gi189017183[0]
Pirital_3945_clone_B_segmentS_gi50981059[7]
Pirital_3945_clone_C_segmentS_gi50981056[14]
Pirital_AV_97021016_segmentS_gi50916222[184]
Pirital_segmentS_gi13274351[24]
Pirital_segmentS_gi48696000[33]
Pirital_VAV-1743_segmentS_gi50981062[187]
Pirital_VAV-1645_segmentS_gi50916210[169]
Whitewater_Arroyo_9310141_segmentS_gi22901302[4]
Whitewater_Arroyo_segmentS_gi188531117[5]
Whitewater_Arroyo_North_American_arenavirusAV_96010024_segment[175]
Whitewater_Arroyo_North_American_arenavirusAV_96010151_segment[157]
Whitewater_Arroyo_North_American_arenavirusAV_D1240007_segment[157]
Catarina_AV_A0400135_segmentS_gi115394823[168]
Catarina_AV_A0400212_segmentS_gi115394826[169]
Skinner_Tank_AV_D1000090_segmentS_gi159461053[168]
Tamiami_W_10777_ands_gi23452251[30]
Tamiami_segmentS_gi188572141[26]
Tamiami_AV_97140103_segmentS_gi186970541[168]
Bear_Canyon_AV_98470029_segmentS_gi78522532[57]
Bear_Canyon_segmentS_gi164607208[40]
Bear_Canyon_A0060209_ands_gi23452267[88]
Latino_Maru_10924_segmentS_gi22901287[6]
Latino_segmentS_gi189026274[11]
Oliveros_segmentS_gi164607193[134]
Chapare_segmentS_gi172088171[145]
Sabia_gi52627079[125]
Cupixi_segmentS_gi164607214[159]
Guanarito_CVH-960101_segmentS_gi45239320[7]
Guanarito_CVH-960102_segmentS_gi50253978[5]
Guanarito_CVH-950801_segmentS_gi50253975[29]
Guanarito_INH-95551_segmentS_gi22901284[0]
Guanarito_segmentS_gi34365538[0]
Guanarito_CVH-960103_segmentS_gi50253972[12]
Guanarito_CVH-960302_segmentS_gi50253969[7]
Guanarito_CVH-961104_segmentS_gi50253987[66]
Guanarito_VHF-3990_segmentS_gi55775478[119]
Guanarito_AV_97021119_segmentS_gi50916219[69]
Guanarito_S-56764_segmentS_gi50253966[65]
Guanarito_VAV-1608_segmentS_gi50253984[137]
Guanarito_VHF-1750_segmentS_gi50253981[127]
Amapari_BeAn_70563_ands_gi23452270[13]
Amapari_segmentS_gi164682561[10]

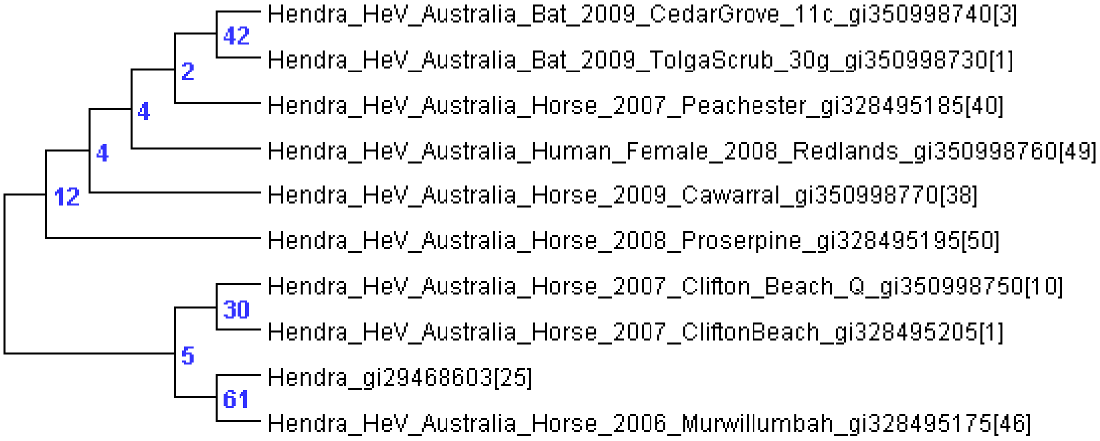
Nipah

Number_SNPs: 684
Number_Homoplastic_SNPs from MSA tree (shown below): 0
Number_Homoplastic_SNPs from SNP Hamming distance tree: 0
Number_Homoplastic_SNPs from SNP RAxML tree: 0
All strains can be uniquely resolved on the basis of SNPs.
Number of SNP microarray probes:
10,573 probes for all alleles
3,090 probes for observed alleles only


Nipah_NV_MY_99_UM-0128_gi40644712[1]
1
Nipah_NV_MY_99_VRI-2794_gi40644698[1]
0
Nipah_UMMC1_gi15487363[1]
43
Nipah_gi13559808[0]
0
Nipah_NV_MY_99_VRI-1413_gi40644705[2]
1
Nipah_UMMC2_gi15487372[2]
0
Nipah_Ind-Nipah-07-FG_from_India_gi253559843[136]
447
Nipah_gi66271892[136]
Nipah_NV_MY_99_VRI-0626_gi57282808[43]

Hendra

Number_SNPs: 437
Number_Homoplastic_SNPs from MSA tree (shown below): 37
Number_Homoplastic_SNPs from SNP Hamming distance tree: 38
Number_Homoplastic_SNPs from SNP RAxML tree: 37
All strains can be uniquely resolved on the basis of SNPs.
Number of SNP microarray probes:
5,841 probes for all alleles
2,280 probes for observed alleles only


Hendra_HeV_Australia_Bat_2009_CedarGrove_11c_gi350998740[3]
42
Hendra_HeV_Australia_Bat_2009_TolgaScrub_30g_gi350998730[1]
2
Hendra_HeV_Australia_Horse_2007_Peachester_gi328495185[40]
4
Hendra_HeV_Australia_Human_Female_2008_Redlands_gi350998760[49]
4
Hendra_HeV_Australia_Horse_2009_Cawarral_gi350998770[38]
12
Hendra_HeV_Australia_Horse_2008_Proserpine_gi328495195[50]
Hendra_HeV_Australia_Horse_2007_Clifton_Beach_Q_gi350998750[10]
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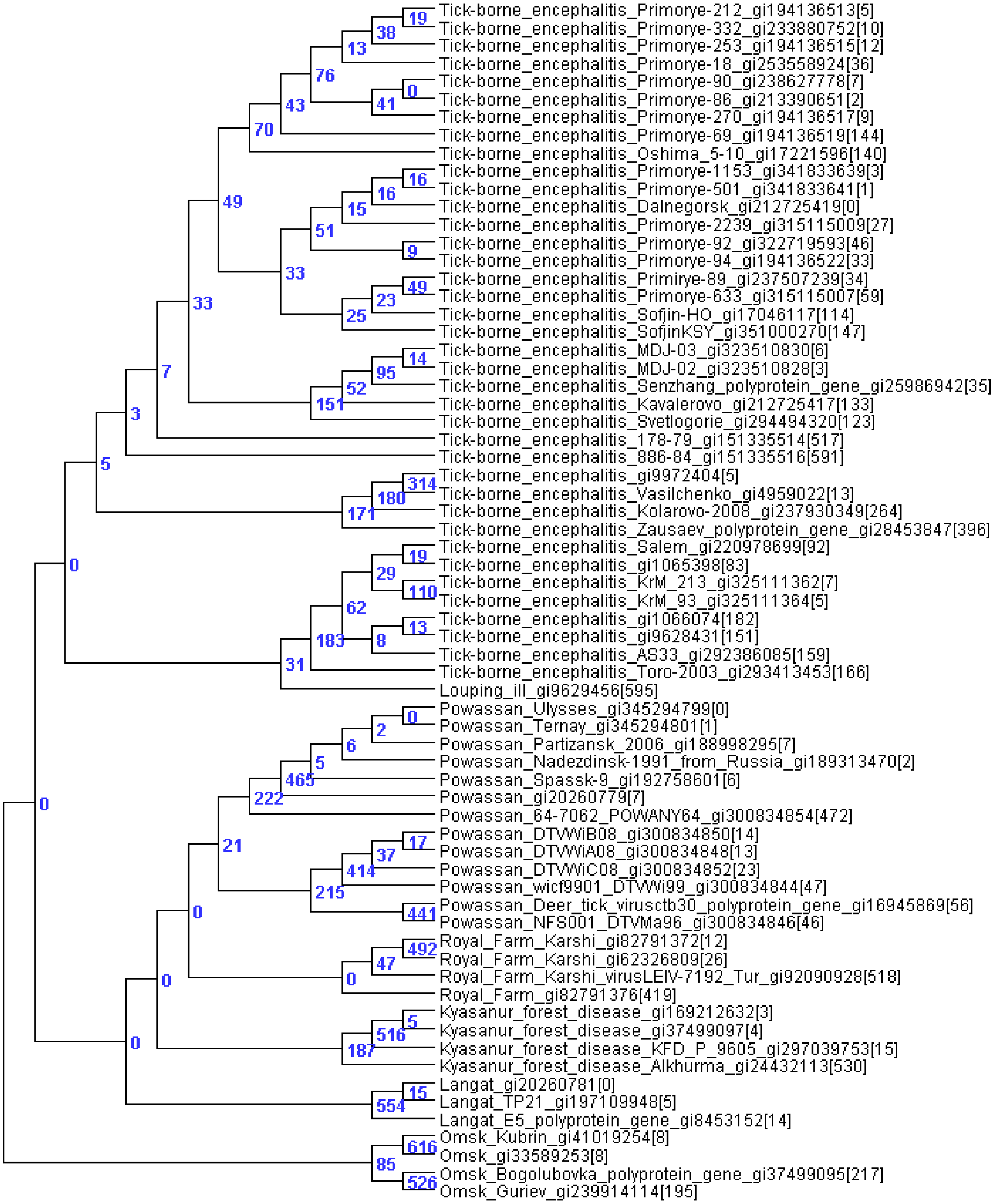
Hendra_HeV_Australia_Horse_2007_CliftonBeach_gi328495205[1]
5
Hendra_gi29468603[25]
61
Hendra_HeV_Australia_Horse_2006_Murwillumbah_gi328495175[46]

Tick-borne encephalitis virus complex

Number_SNPs: 10,276
Number_Homoplastic_SNPs from MSA tree (shown below): 1260
Number_Homoplastic_SNPs from SNP Hamming distance tree: 1272
Number_Homoplastic_SNPs from SNP RAxML tree: 1759
All strains can be uniquely resolved on the basis of SNPs.
Number of SNP microarray probes:
288,269 probes for all alleles
78,225 probes for observed alleles only


Tick-borne_encephalitis_Primorye-212_gi194136513[5]
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Tick-borne_encephalitis_Primorye-332_gi233880752[10]
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Tick-borne_encephalitis_Primorye-253_gi194136515[12]
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Tick-borne_encephalitis_Primorye-18_gi253558924[36]
76
Tick-borne_encephalitis_Primorye-90_gi238627778[7]
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41
43
Tick-borne_encephalitis_Primorye-270_gi194136517[9]
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Tick-borne_encephalitis_Primorye-69_gi194136519[144]
Tick-borne_encephalitis_Oshima_5-10_gi17221596[140]
Tick-borne_encephalitis_Primorye-1153_gi341833639[3]
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Tick-borne_encephalitis_Primorye-501_gi341833641[1]
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Tick-borne_encephalitis_Dalnegorsk_gi212725419[0]
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Tick-borne_encephalitis_Primorye-92_gi322719593[46]
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Tick-borne_encephalitis_Primorye-94_gi194136522[33]
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Tick-borne_encephalitis_Primirye-89_gi237507239[34]
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Tick-borne_encephalitis_Primorye-633_gi315115007[59]
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Tick-borne_encephalitis_Sofjin-HO_gi17046117[114]
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Tick-borne_encephalitis_SofjinKSY_gi351000270[147]
Tick-borne_encephalitis_MDJ-03_gi323510830[6]
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Tick-borne_encephalitis_MDJ-02_gi323510828[3]
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Tick-borne_encephalitis_Svetlogorie_gi294494320[123]
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Tick-borne_encephalitis_886-84_gi151335516[591]
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Tick-borne_encephalitis_Zausaev_polyprotein_gene_gi28453847[396]
Tick-borne_encephalitis_Salem_gi220978699[92]
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Tick-borne_encephalitis_KrM_213_gi325111362[7]
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Tick-borne_encephalitis_gi1066074[182]
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Tick-borne_encephalitis_AS33_gi292386085[159]
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Tick-borne_encephalitis_Toro-2003_gi293413453[166]
Louping_ill_gi9629456[595]
Powassan_Ulysses_gi345294799[0]
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Powassan_Ternay_gi345294801[1]
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Powassan_Partizansk_2006_gi188998295[7]
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Powassan_Nadezdinsk-1991_from_Russia_gi189313470[2]
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Powassan_Spassk-9_gi192758601[6]
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Powassan_gi20260779[7]
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Powassan_64-7062_POWANY64_gi300834854[472]
Powassan_DTVWiB08_gi300834850[14]
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Powassan_DTVWiA08_gi300834848[13]
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Powassan_DTVWiC08_gi300834852[23]
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Powassan_wicf9901_DTVWi99_gi300834844[47]
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Powassan_Deer_tick_virusctb30_polyprotein_gene_gi16945869[56]
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Royal_Farm_Karshi_gi82791372[12]
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Langat_gi20260781[0]
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Omsk_gi33589253[8]
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Omsk_Bogolubovka_polyprotein_gene_gi37499095[217]
526
Omsk_Guriev_gi239914114[195]

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