Constancy and Change in the 5'UTR of Yellow Fever Virus

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Abstract

The nucleotide composition of the 5'UTR of the yellow fever virus (YFV) has been reported to be the most constant sequence in the viral genome, but a comprehensive analysis of this constancy has not been presented. The current report is an analysis of the 5'UTRs from 48 sequences deposited in GenBank representing the seven described genotypes, five in Africa and two in the Americas. The YFV 5'UTRs consist of 118-120 nucleotides, 92% (110/119) of which are constant in all sequences. The constancy is impressive and suggests that many participate in significant viral functions. Remarkably, analysis of the non-constant nucleotides revealed that in some instances the non-constant nucleotide changes persisted in one or a restricted number of related genotypes and were from sequences isolated over a considerable span of years. This constant feature of non-constant nucleotides is consistent with the concept that the changes were in response to different environmental features such as changes in mosquito hosts or animal reservoirs, particularly as a consequence of spread of YFV from Africa to the New World. Constancy of 5'UTR in general may be helpful in distinction of viral species. Lastly, the presence of sequences of constant nucleotides greater than 19 nucleotides suggests regions of the 5'UTR that may be exploited for use as non-codon RNA as treatment and diagnostic agents in a variety of viral diseases.

Importance

The 5'UTR is arguably the most neglected portion of the viral genome. It is frequently incomplete in the sequences deposited as otherwise complete sequences in GenBank. The current report is an analysis of complete 5'UTR sequences selected from those deposited in GenBank and indicates that the 5'UTR is 92% conserved confirming that it is a highly conserved portion of the viral genome and suggesting that each conserved nucleotide may be functionally significant. Repeated occurrences of even non-constant nucleotides belong to a restricted number of genotypes raising the possibility that adaptation to new mosquito hosts and animal reservoirs such as those that accompanied spread of yellow fever virus from Africa to the Western Hemisphere are relevant. Knowledge of prolonged strings of invariable nucleotides in the 5'UTR

has been used in designing a method for detecting YFV and may also be relevant for designing sequences for viral control of a variety of viruses.

Introduction

YFV is the eponymous virus for the genus Flavivirus. Originally isolated in 1927 from a patient in what is now Ghana, the nucleotide sequence of the vaccine virus derived from YFV was reported in 1985 (1). YFV persists as the cause jungle yellow fever transmitted by various mosquito species (*Anopheles sp.* in Africa and by *Haemagogus* sp. and *Sabethes* sp. in the Americas). Although detailed observations have not been presented, non-human primates (NHPs) in Africa are thought to be resistant to fatal infection (2). In the Americas, some NHPs succumb to the virus (e.g. *Alouatta* sp. (howler monkeys) (3) and *Sapajus* sp (capuchins) (4), but others are usually resistant to lethal infection (e.g. *Callithrix* sp (marmosets) (5) and Leontopithecus sp (golden lion tamarins) (6)). In both Africa and the Americas, the feared complication of yellow fever is the development of urban yellow fever in which the virus is rapidly spread amongst humans by *Aedes aegypti* and is associated with a high human mortality rate.

YFV has a 5'UTR, a sequence (cds) encoding three structural and seven non-structural proteins, and a 3'UTR. The virus originated in Africa and spread to the Americas in the 17th century in ships bearing slaves (7). Currently the virus persists in sub-Saharan Africa and in South America. Five genotypes are recognized in Africa and two in S. America (8). The genotypes are associated with different geographical regions. More recently sub-clade lineages of the S. American genotypes have become recognized (9). As with other viruses that lack a proof-reading mechanism in their RNA polymerases, mutations are common. Accordingly, considerable sequence diversity is anticipated.

The current report is an analysis of the YFV 5'UTR sequences deposited in GenBank. They were isolated from the seven genotypes (Table 1) . Three aspects are evaluated: 1) Nucleotide constancy, 2) an analysis of constant features of non-constant nucleotides, and 3) suggestions are presented for further exploration. The results demonstrate that YFV 5'UTR consists of 118-120 highly conserved (92%) nucleotides and is much more conserved than nucleotides in the cds or the 3'UTR (10, 11), Seligman, S.J. unpublished).

The relative constancy of YFV 5'UTR has been used in the development of a method to detect the YFV genome in point of care facilities and reference laboratories (11). Since YFV and other flaviviruses are noted for their nucleotide variability, the constant nucleotides in the 5'UTR suggest that each may have a raison d'etre. Analysis of some of the few non-constant YFV nucleotides reveals that they can persist for prolonged periods and be associated with a single genotype suggesting that there is a survival advantage for them as well to the mutation possibly related to different species of mosquito hosts and/or NHPs associated with particular geographic areas in which they propagate in South America (Table 2).

To evaluate the extent to which constant yellow fever 5'UTR nucleotides remained constant in other flaviviruses, a similar analysis was done of 40 Zika virus 5'UTR sequences (data not shown). The Zika sequences contained 106-107 nucleotides, 90 of which were constant (84%).

In contrast with the non-constant 5'UTR YFV nucleotides, those of Zika virus were found in more recent isolates reflecting the explosive Zika epidemic. Whether the non-constant Zika nucleotides persist depends on future propagation of the virus.

The current analysis of constant and non-constant YFV 5'UTR nucleotides has the potential for yielding clues relating to the definition of virus species, the phenotypic implications of specific nucleotides, and the identification of stretches of conserved viral nucleotides helpful for the development of treatment non-coding RNAs.

Methods

A search for suitable YFV sequences were made in GenBank, (https://www.ncbi.nlm.nih.gov/genbank/), an annotated collection of all publicly available DNA sequences. Search was made for sequences originating from different countries in Africa or Latin America in which yellow fever is or had been occurring. Sequences in which the 5' end of the 5'UTR was incomplete were omitted. An alignment of 48 sequences was done. Aligned columns were evaluated in an effort to evaluate whether unique instances of non-constant nucleotides represented sequencing error or failure of these mutations to persist. Two such instances were found, a 1935 Brazilian isolate (U52389) and a Chinese isolate from a case acquired in Angola (KU921608) in 2016. In U52389 two different non-constant nucleotides and in KU921608 three occurred. A search using Blastn (https://blast.ncbi.nlm.nih.gov/) confirmed that the nucleotide changes in these two isolates were unique. The lack of repeat instances of these nucleotides in the relevant positions is consistent with either sequencing error or failure of the mutation to persist on subsequent viral replication thereby indicating that it is not an essential 5'UTR nucleotide, at least under the condition in which the virus was propagating.

Results

Alignment of the 5'UTRs from 48 yellow fever strains including representatives from all of the seven recognized genotypes (8, 12, 13) indicates that their lengths varied from 118-120 nucleotides, 110 of which remained constant (92%) (Table 1). In contrast, 65% (6655/10230) of cds and 58% (266/459) of 3'UTR nucleotides were invariable (data not shown). In the case of cds variations, an increase in nucleotide constancy was noted with increasing size of the predicted viral protein (data not shown). The 3'UTR estimate is based on 22 isolates (14)). Sub-genomic stratification of repeated nucleotide sequence elements in the 3'UTR found in some YFV genotypes was not included in the calculations. Consequently more nuanced evaluations using sub-genomic sequences of the 3'UTR might increase the prevalence of constant nucleotides. The results do not necessarily establish all of the 19 or so consecutive conserved nucleotides necessary for viral growth inhibition by potential varieties of interfering RNA. The establishment of the combination of such nucleotides for the entire viral genome is beyond the scope of this manuscript.

Analysis of the non-constant nucleotides revealed two distinct patterns (Tables 1 and 2). In some instances (nucleotides 81, 89, 118, and 119), the non-constant nucleotides persisted for many decades in a single genotype suggesting a survival advantage of the relevant YFV in a particular geographic region that contained a specific mosquito species and/or non-human primate host.

Instances of the occurrence of a given nucleotide change or changes in one or two sequences could be the result sequencing error. In any event failure of the nucleotide change to persist suggests minimal effect of their ability to confer a significant advantage to viral propagation.

Conclusions

Analysis of constant and non-constant nucleotides in the 5'UTR of yellow fever virus that their persistence over the course of decades suggests that at least some nucleotides play a significant role in viral defense. Restraints in nucleotide changes in this region are likely to be of considerable interest. What mechanisms might be involved? In recent years considerable attention has been placed investigating the role of non-coding RNA in viral infections. Nucleotide sequences longer than 200 are usually considered as long non-coding RNA and have been found to be important both in host cell response and virus defense mechanisms (15, 16). In addition small non-coding RNA's (RNAi) have a variety of formulations including both single-stranded RNA (micro (mi)RNA) (17) and double-stranded RNA (siRNA).Their actions may be promulgated either in *cis* or in *trans* (sfRNA) (18). Piwi-interacting (pi)RNA are found only in the testes and are involved in suppressing transposons (19).

Which, if any of these non-codon RNAs, are present in the 5'UTR is conjectural. Although 5'UTR nucleotides in alphaviruses have been implicated in host defense (20), the composition of RNAs in the 5'UTR that might be involved needs to be investigated systematically. Modern editing techniques changing selected 5'UTR nucleotides should be explored to investigate phenotypic changes in viral properties. Of particular interest is the possibility that nucleotide sequences from the 5'UTR are crucial in enabling YFV to propagate in primates and/or mosquitoes. Although no experiments have thus far reported systematically investigating the constancy of nucleotides in the 5'UTR, the effectiveness of the varieties of small interfering RNA in the control of virus infection is a field of active investigation (21-25). Should suitable conditions be found in which 5'UTR nucleotide sequences are determined to be inhibitory to YFV, parenteral delivery methods of the relevant inhibitory nucleotides would need to be developed to achieve a therapeutic effect. Most intriguing is the possibility that these concepts may also be applicable to other viruses such as coronaviruses, an idea of special significance in the control of rapidly emerging pandemics.

Supplemental Material

none

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Table 1. Alignment of yellow fever virus 5'UTR

YF_14FA_AY968064_von_Lindern_Angola_human_1971	agtaaatccctgggtgctaattgaggtgcattggtctgcaaatcgagttgctaggcaata	60
YF_CIC1_KX010994_Pan_China_exAngola_human_blood_2016	agtaaatccctgggtgctaattgaggtgcattggtctgcaaatcgagttgctaggcaata	60
YF_CIC4_KX027336_Cui_China_exAngola_human_urine_2016	agtaaatccctgggtgctaattgaggtgcattggtctgcaaatcgagttgctaggcaata	60
YF_Uganda-2010_JN620362_McMullan_Uganda_East/Central-Africa_human_2010	agtaaatc-ctgtgtgctaattgaggtgcattggtctgcaaatcgagttgctaggcaata	59
YF_A7094A2_AY968065_Von_Lindern_East-Africa_Uganda48a_1948	agtaaatc-ctgtgtgctaattgaggtgcattggtctgcaaatcgagttgctaggcaata	59
YF_M_90-5_TVP_3230_MF004383_Baronti_Sudan_East-Africa_human_serum<2017	agtaaatc-ctgtgtgctaattgaggtgcattggtctgcaaatcgagttgctaggcaata	59
YF_Couma_DQ235229_von_Lindern_Ethiopia_East/Central-Africa_human_1961	agtaaatc-ctgtgtgctaattgaggtgcattggtctgcaaatcgagttgctaggcaata	59
YF_Uga48_U52422_Wang_Uganda_East-Africa_1948	agtaaatc-ctgtgtgctaattgaggtgcattggtctgcaaatcgagttgctaggcaata	59
YF_Car77(900)_U52395_Wang_Central-African-Republic_East/Central-Africa_1977	agtaaatc-ctgtgtgctaattgaggtgcattggtctgcaaatcgagttgctaggcaata	59
YF_Car77(883)_U52392_Wang_Central-African-Republic_East/Central-Africa_1977	agtaaatc-ctgtgtgctaattgaggtgcattggtctgcaaatcgagttgctaggcaata	59
YF_85-82H_U54798_Pisano_Ivory-Coast_West-Africa-I_human_1982	agtaaatc-ctgtgtgctaattgaggtgcattggtctgcaaatcgagttgctaggcaata	59
YF_Ivory-Coast-1999_AY603338_Bae_Ivory-Coast_West-Africa-I_1999	agtaaatc-ctgtgtgctaattgaggtgcattggtctgcaaatcgagttgctaggcaata	59
YF_DakArAmt7_JX898869_Stock_Ivory-Coast_West-Africa_II_1973	agtaaatc-ctgtgtgctaattgaggtgcattggtctgcaaatcgagttgctaggcaata	59
YF_ArD181439_JX898881_Stock_Senegal_West-Africa-II_2005	agtaaatc-ctgtgtgctaattgaggtgcattggtctgcaaatcgagttgctaggcaata	59
YF_ArD181564_JX898880_Stock_Senegal_West-Africa-II_2005	agtaaatc-ctgtgtgctaattgaggtgcattggtctgcaaatcgagttgctaggcaata	59
YF_HD117294_JX898868_Stock_Senegal_West-Africa-II_human_1995	agtaaatc-ctgtgtgctaattgaggtgcattggtctgcaaatcgagttgctaggcaata	59
YF_Gambia01_AY572535_Bae_Gambia_West-Africa-II_human_2001	agtaaatc-ctgtgtgctaattgaggtgcattggtctgcaaatcgagttgctaggcaata	59
YF_Asibi_AY640589_McElroy_Ghana_West-Africa-II_human_1927	agtaaatc-ctgtgtgctaattgaggtgcattggtctgcaaatcgagttgctaggcaata	59
YF_Trinidad79P_AF094612_Pisano_Trinidad_West_African_II_1979	agtaaatc-ctgtgtgctaattgaggtgcattggtctgcaaatcgagttgctaggcaata	59
YF_VL2926_MK333809_Delatorre_Brazil-Valenca-RJ_S_America_I_2018	agtaaatc-ctgggtgctaattgaggtgcattggtctgcaaatcgagttgctaggcaata	59
YF_BeAR513008_JF912185_Nunes_Brazil_S_America_I_Sabethes-sp_1992	agtaaatc-ctgtgtgctaattgaggtgcattggtctgcaaatcgagttgctaggcaata	59
YF_H196_MF538784_Gomez_Brazil_S-American-I_Porciuncula-RJ_2017	agtaaatc-ctgtgtgctaattgaggtgcattggtctgcaaatcgagttgctaggcaata	59
YF_GO21_MK728873_Delatorre_Brazil_S-American-I_Amorinopolis-GO_2017	agtaaatc-ctgtgtgctaattgaggtgcattggtctgcaaatcgagttgctaggcaata	59
YF_BeH622205_JF912187_Nunes_Brazil_S-American-I_2000	agtaaatc-ctgtgtgctaattgaggtgcattggtctgcaaatcgagttgctaggcaata	59
YF_Panama74_U52404_Wang_Panama_S_America_I_1974	agtaaatc-ctgtgtgctaattgaggtgcattggtctgcaaatcgagttgctaggcaata	59
YF_RJ-155_MK533792_Abreu_Brazil_Casimiro-de-Abreu_S_America_I_2019	agtaaatc-ctgtgtgctaattgaggtgcattggtctgcaaatcgagttgctaggcaata	59
YF_RJ97_MF538785_Gomez_Brazil_Araras_S_America_I_marmoset_2017	agtaaatc-ctgtgtgctaattgaggtgcattggtctgcaaatcgagttgctaggcaata	59
YF_PA196_MF423374_Gomez_Brazil_Paraju_S_America_I_2017	agtaaatc-ctgtgtgctaattgaggtgcattggtctgcaaatcgagttgctaggcaata	59
YF_RJPR4867_MF370548_Filippis_Brazil_S_America_I_liver_2017	agtaaatc-ctgtgtgctaattgaggtgcattggtctgcaaatcgagttgctaggcaata	59
YF_PA193_MF423373_Gomez_Brazil_Paraju_S_America_I_2017	agtaaatc-ctgtgtgctaattgaggtgcattggtctgcaaatcgagttgctaggcaata	59
YF_ES-504_KY885000_Bonaldo_Brazil-Domingos-Martins-ES_S_Americoca_I_2017	agtaaatc-ctgtgtgctaattgaggtgcattggtctgcaaatcgagttgctaggcaata	59
YF_H191_MF538783_Gomez_Brazil-Casimiro-de-Abreu-RJ_S_America_I_2017	agtaaatc-ctgtgtgctaattgaggtgcattggtctgcaaatcgagttgctaggcaata	59
YF_H189_MG550109_Gomez_Brazil-Bananal-Marica-RJ_S_America_I_2017	agtaaatc-ctgtgtgctaattgaggtgcattggtctgcaaatcgagttgctaggcaata	59
YF_H190_MF538782_Gomez_Brazil-Sao-Fidelis-RJ_S_America_I_human_2017	agtaaatc-ctgtgtgctaattgaggtgcattggtctgcaaatcgagttgctaggcaata	59

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YF_PR5952-BeAn845405_MF370544_Cruz_Brazil_S_America_I_liver_2017          agtaaatc-ctgtgtgctaattgaggtgcattggtctgcaaatcgagttgctaggcaata  59
YF_PR5937-BeAn845130_MF370543_Cruz_Brazil_S_America_I_liver_2017          agtaaatc-ctgtgtgctaattgaggtgcattggtctgcaaatcgagttgctaggcaata  59
YF_MG3155_MK333808_Delatorre_Brazil_Belmiro-Braga-MG_S_America_I_2018     agtaaatc-ctgtgtgctaattgaggtgcattggtctgcaaatcgagttgctaggcaata  59
YF_MG3121_MK333807_Delatorre_Brazil_Belmiro-Braga-MG_S_America_I_2018     agtaaatc-ctgtgtgctaattgaggtgcattggtctgcaaatcgagttgctaggcaata  59
YF_IG3036_MK333805_Delatorre¬_Brazil-Ilha-Grande-RJ_S_America_I_2018      agtaaatc-ctgtgtgctaattgaggtgcattggtctgcaaatcgagttgctaggcaata  59
YF_BeAR646536_JF912189_Nunes_Brazil_S._America_I_2001                     agtaaatc-ctgtgtgctaattgaggtgcattggtctgcaaatcgagttgctaggcaata  59
YF_BeAR378600_JF912179_Nunes_Brazil_S._America_I_1980                     agtaaatc-ctgtgtgctaattgaggtgcattggtctgcaaatcgagttgctaggcaata  59
YF_ES2750_MK333802_Delatorre_Brazil-Santa-Leopoldina-ES_S_America_I_2017  agtaaatc-ctgtgtgctaattgaggtgcattggtctgcaaatcgagttgctaggcaata  59
YF_88/1999_MF004382_Baronti_Bolivia_S_American_II_human_serum_1999        agtaaatc-ctgtgtgctaattgaggtgcattggtctgcaaatcgagttgctaggcaata  59
YF_Trinidad79W_U52419_Wang_Trinidad_S_America_II_1979                     agtaaatc-ctgtgtgctaattgaggtgcattggtctgcaaatcgagttgctaggcaata  59
YF_Peru95(153)_U52410_Wang_Brazil_S_America_II_1995                       agtaaatc-ctgtgtgctaattgaggtgcattggtctgcaaatcgagttgctaggcaata  59
YF_Peru95(149)_U52409_Wang_Brazil_S_America_II_1995                       agtaaatc-ctgtgtgctaattgaggtgcattggtctgcaaatcgagttgctaggcaata  59
YF_Trinidad54_U52416_Wang_Trinidad_S_America_I_1954                       agtaaatc-ctgtgtgctaattgaggtgcattggtctgcaaatcgagttgctaggcaata  59
YF_Ecuador79_U52398_Wang_Ecuador_S_America_I_1979                         agtaaatc-ctgtgtgctaattgaggtgcattggtctgcaaatcgagttgctaggcaata  59
                                                                          ******** *** **********************************************

YF_14FA_AY968064_von_Lindern_Angola_human_1971                            aacacatttggattaattttaatcgttcgttgagcgattagcagagaactgaccagagaa  120
YF_CIC1_KX010994_Pan_China_exAngola_human_blood_2016                      aacacatttggattaattttaatcgttcgttgagcgattagcagagaactgaccagagaa  120
YF_CIC4_KX027336_Cui_China_exAngola_human_urine_2016                      aacacatttggattaattttaatcgttcgttgagcgattagcagagaactgaccagagaa  120
YF_Uganda-2010_JN620362_McMullan_Uganda_East/Central-Africa_human_2010    aacacatttggattaattttaatcgttctttgagcgattagcagagaactgaccagagta  119
YF_A7094A2_AY968065_Von_Lindern_East-Africa_Uganda48a_1948                aacacatttggattaattttaatcgttctttgagcgattagcagagaactgaccagaaaa  119
YF_M_90-5_TVP_3230_MF004383_Baronti_Sudan_East-Africa_human_serum<2017    aacacatttggattaattttaatcgttctttgagcgattagcagagaactgaccagaaaa  119
YF_Couma_DQ235229_von_Lindern_Ethiopia_East/Central-Africa_human_1961     aacacatttggattaattttaatcgttctttgagcgattagcagagaactgaccagaaaa  119
YF_Uga48_U52422_Wang_Uganda_East-Africa_1948                              aacacatttggattaattttaatcgttctttgagcgattagcagagaactgaccagaaa-  118
YF_Car77(900)_U52395_Wang_Central-African-Republic_East/Central-Africa_1977  aacacatttggattaattttaatcgttctttgagcgattagcagagaactgaccagaac-  118
YF_Car77(883)_U52392_Wang_Central-African-Republic_East/Central-Africa_1977  aacacatttggattaattttaatcgttctttgagcgattagcagagaactgaccagaac-  118
YF_85-82H_U54798_Pisano_Ivory-Coast_West-Africa-I_human_1982              aacacatttggattaattttaatcgttcgttgagcgattagcagagaactgaccagaat-  118
YF_Ivory-Coast-1999_AY603338_Bae_Ivory-Coast_West-Africa-I_1999           aacacatttggattaattttaatcgttcgttgagcgattagcagagaactgaccagaac-  118
YF_DakArAmt7_JX898869_Stock_Ivory-Coast_West-Africa_II_1973               aacacatttggattaattttaatcgttcgttgagcgattagcagagaactgaccagaac-  118
YF_ArD181439_JX898881_Stock_Senegal_West-Africa-II_2005                   aacacatttggattaattttaatcgttcgttgagcgattagcagagaactgaccagaac-  118
YF_ArD181564_JX898880_Stock_Senegal_West-Africa-II_2005                   aacacatttggattaattttaatcgttcgttgagcgattagcagagaactgaccagaac-  118
YF_HD117294_JX898868_Stock_Senegal_West-Africa-II_human_1995              aacacatttggattaattttaatcgttcgttgagcgattagcagagaactgaccagaac-  118
YF_Gambia01_AY572535_Bae_Gambia_West-Africa-II_human_2001                 aacacatttggattaattttaatcgttcgttgagcgattagcagagaactgaccagaac-  118
YF_Asibi_AY640589_McElroy_Ghana_West-Africa-II_human_1927                 aacacatttggattaattttaatcgttcgttgagcgattagcagagaactgaccagaac-  118
YF_Trinidad79P_AF094612_Pisano_Trinidad_West_African_II_1979              aacacatttggattaattttaatcgttcgttgagcgattagcagagaactgaccagaac-  118
YF_VL2926_MK333809_Delatorre_Brazil-Valenca-RJ_S_America_I_2018           aacacatttggattaattttgatcgttcgttgagcgattagcagagaattgaccagaaa-  118
YF_BeAR513008_JF912185_Nunes_Brazil_S_America_I_Sabethes-sp_1992          aacacatttggattaattttgatcgttcgttgagcgattagcagagaactgaccagaaa-  118
YF_H196_MF538784_Gomez_Brazil_S-American-I_Porciuncula-RJ_2017            aacacatttggattaattttgatcgttcgttgagcgattagcagagaattgaccagaaa-  118
YF_GO21_MK728873_Delatorre_Brazil_S-American-I_Amorinopolis-GO_2017       aacacatttggattaattttgatcgttcgttgagcgattagcagagaattgaccagaaa-  118
YF_BeH622205_JF912187_Nunes_Brazil_S-American-I_2000                      aacacatttggattaattttgatcgttcgttgagcgattagcagagaattgaccagaaa-  118
YF_Panama74_U52404_Wang_Panama_S_America_I_1974                           aacacatttggattaattttgatcgttcgttgagcgattagcagagaattgaccagaaa-  118
YF_RJ-155_MK533792_Abreu_Brazil_Casimiro-de-Abreu_S_America_I_2019        aacacatttggattaattttgatcgttcgttgagcgattagcagagaattgaccagaaa-  118
YF_RJ97_MF538785_Gomez_Brazil_Araras_S_America_I_marmoset_2017            aacacatttggattaattttgatcgttcgttgagcgattagcagagaattgaccagaaa-  118
YF_PA196_MF423374_Gomez_Brazil_Paraju_S_America_I_2017                    aacacatttggattaattttgatcgttcgttgagcgattagcagagaattgaccagaaa-  118
YF_RJPR4867_MF370548_Filippis_Brazil_S_America_I_liver_2017               aacacatttggattaattttgatcgttcgttgagcgattagcagagaattgaccagaaa-  118
YF_PA193_MF423373_Gomez_Brazil_Paraju_S_America_I_2017                    aacacatttggattaattttgatcgttcgttgagcgattagcagagaattgaccagaaa-  118
YF_ES-504_KY885000_Bonaldo_Brazil-Domingos-Martins-ES_S_Amerioca_I_2017   aacacatttggattaattttgatcgttcgttgagcgattagcagagaattgaccagaaa-  118
YF_H191_MF538783_Gomez_Brazil-Casimiro-de-Abreu-RJ_S_America_I_2017       aacacatttggattaattttgatcgttcgttgagcgattagcagagaattgaccagaaa-  118
YF_H189_MG550109_Gomez_Brazil-Bananal-Marica-RJ_S_America_I_2017          aacacatttggattaattttgatcgttcgttgagcgattagcagagaattgaccagaaa-  118
YF_H190_MF538782_Gomez_Brazil-Sao-Fidelis-RJ_S_America_I_human_2017       aacacatttggattaattttgatcgttcgttgagcgattagcagagaattgaccagaaa-  118
YF_PR5952-BeAn845405_MF370544_Cruz_Brazil_S_America_I_liver_2017          aacacatttggattaattttgatcgttcgttgagcgattagcagagaattgaccagaaa-  118
YF_PR5937-BeAn845130_MF370543_Cruz_Brazil_S_America_I_liver_2017          aacacatttggattaattttgatcgttcgttgagcgattagcagagaattgaccagaaa-  118
YF_MG3155_MK333808_Delatorre_Brazil_Belmiro-Braga-MG_S_America_I_2018     aacacatttggattaattttgatcgttcgttgagcgattagcagagaattgaccagaaa-  118
YF_MG3121_MK333807_Delatorre_Brazil_Belmiro-Braga-MG_S_America_I_2018     aacacatttggattaattttgatcgttcgttgagcgattagcagagaattgaccagaaa-  118
YF_IG3036_MK333805_Delatorre¬_Brazil-Ilha-Grande-RJ_S_America_I_2018      aacacatttggattaattttgatcgttcgttgagcgattagcagagaattgaccagaaa-  118
YF_BeAR646536_JF912189_Nunes_Brazil_S._America_I_2001                     aacacatttggattaattttgatcgttcgttgagcgattagcagagaattgaccagaaa-  118
YF_BeAR378600_JF912179_Nunes_Brazil_S._America_I_1980                     aacacatttggattaattttgatcgttcgttgagcgattagcagagaattgaccagaaa-  118
YF_ES2750_MK333802_Delatorre_Brazil-Santa-Leopoldina-ES_S_America_I_2017  aacacatttggattaattttgatcgttcgttgagcgattagcagagaattgaccagaaa-  118
YF_88/1999_MF004382_Baronti_Bolivia_S_American_II_human_serum_1999        aacacatttggattaattttgatcgttcgttgagcgattagcagagaactgaccagata-  118
YF_Trinidad79W_U52419_Wang_Trinidad_S_America_II_1979                     aacacatttggattaattttgatcgttcgttgagcgattagcagagaactgaccagata-  118
YF_Peru95(153)_U52410_Wang_Brazil_S_America_II_1995                       aacacatttggattaattttgatcgttcgttgagcgattagcagagaactgaccagata-  118
YF_Peru95(149)_U52409_Wang_Brazil_S_America_II_1995                       aacacatttggattaattttgatcgttcgttgagcgattagcagagaactgaccagata-  118
YF_Trinidad54_U52416_Wang_Trinidad_S_America_I_1954                       aacacatttggattaattttgatcgttcgttgagcgattagcagagaattgaccagcac-  118
YF_Ecuador79_U52398_Wang_Ecuador_S_America_I_1979                         aacacatttggattaattttgatcgttcgttgagcgattagcagagaattgaccagcta-  118
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Table 2. Non-constant 5'UTR nucleotides in 48 yellow fever virus sequences

Nucleotide number	Nucleotide change	Genotypes with changed nucleotide		
9	a insertion	Angolan		
13	g13t	Angolan		
81	g81a	S. American I, Angolan		
89	g89t	East Central Africa		
109	c109t	S. American I		
117	a117c	2 sequences S. American I From 1954 and 1971		
118	t118c	S. American II and 1 sequence from S. American I		
119	a119c	West Africa II, 2 sequences from East/Central-Africa_1977, and 1 sequence from S_America_I_1954		
119	a119t	1 sequence from East/Central-Africa_human_2010 and 1 sequence from West-Africa-I_1982		

120	a 120 deletion	An artifact of the alignment program. The deleted “a” is more reasonably considered to be the initial “a” in atg in the ensuing cds.		