

Supplemental materials

Supplemental Table 1. Primers for cloning ZIKV mini-replicons

Primer name	Primer sequences
ZIKV 5'UTR-C-eGFP-Δ-RdRp-3'UTR (ZV mini-rep 1-GFP)	
NotI_Rsr_ZV nt7830-For	5'-CGCGGCCGCACGGACCGGTGTCCCGAGGAAGTGCAAAGCTGAGATG-3'
ZV C_KpnI_AscI-Rev	5'-GTTGGCGCGCCGGTACCTGCACTCCCACGTCTAGTGAC-3'
AscI_T2A_GFP-For	5'-CCGGCGCGCCAACCGGTACGCGTGAGGGCAGGGGAAGTCTTCTAACATGCGGGGACGTGGAGGAAAATCCCGGGCCCATGGTGAGCAAGGCGAGGAGC-3'
F2A_NotI-Rev	5'-CCCTGCGGCCGCGGGCCCCGGGGTTGGA-3'
ZIKV 5'UTR-C-eGFP-Δ-NS5-3'UTR (ZV mini-rep 2-GFP)	
ZIKV NS5 MTase_NotI-For	5'-ACCGCGGCCGCAGGGGGTGGAACAGGAGAGACCTG-3'
ZIKV NS5(8406)_AvrII-Rev	5'-TATTTCACTGGCCTCCTAGGCCCGT-3'
ZIKV 5'UTR-C-eGFP-Δ-NS4A'-NS4B-NS5-3'UTR (ZV mini-rep 3-GFP)	
NS4A'_NotI For	5'-ATAGCGGCCGCATTGGGCAAGGGCATAGGG-3'
ZIKV 5'UTR-C-Δ-RdRp-3'UTR (ZV mini-rep 1-no reporter)	
ClaI_EcoRV_3'GFP-For	5'-GTATCGATGATATCACTCTCGGCATGGACGAG-3'
5'GFP_MluI_ClaI-Rev	5'-TCATCGATACGCGTGAACAGCTCCTCGCCCTT-3'
ZIKV 5'UTR-C-CFP/YFP-Δ-RdRp-3'UTR (ZV mini-rep 1-CYP)	
KpnI_CFP-For	5'-ATAGGTACCATGGTTAGCAAGGGCGAG-3'
YFP_AscI-Rev	5'-TATGGCGCGCCGTTTGTACAGTTCGTCCAT-3'
ZIKV 5'UTR-C-CFP/YFP-Δ-NS5-3'UTR (ZV mini-rep 2-CYP)	
ZIKV NS5 MTase_NotI-For	5'-ACCGCGGCCGCAGGGGGTGGAACAGGAGAGACCTG-3'
ZIKV NS5(8406)_AvrII-Rev	5'-TATTTCACTGGCCTCCTAGGCCCGT-3'
ZIKV 5'UTR-C-CFP/YFP-Δ-NS4A'-NS4B-NS5-3'UTR (ZV mini-rep 3-CYP)	
NS4A'_NotI For	5'-ATAGCGGCCGCATTGGGCAAGGGCATAGGG-3'
ZIKV 5'UTR-C-hACE2-eGFP-Δ-NS5-3'UTR (ZV mini-rep 2-hACE2)	
KpnI-ACE2-For	5'-ATAGGTACCATGTCAAGCTCTTCCTGG-3'
ACE2_AscI-Rev	5'-ATAGGCGCGCCTTTACCCGGAGACAGGGAAG-3'

Supplemental Table 2. Primers for real-time RT PCR assays

Primer name	Primer sequences
ZIKV NS5	
ZV-NS5-For	5'-CTTGTGGCTGCTGCGGAGGTCA-3'
ZV-NS5-Rev	5'-GTGGTGGGAGCAAACGGAAC TT-3'
Human ACE2	
hACE2-For	5'-AGCCCCCATCGAGAAAACCATC-3'
Hace2-Rev	5'-TGCCCAT TGCTCTCCCACTCC-3'

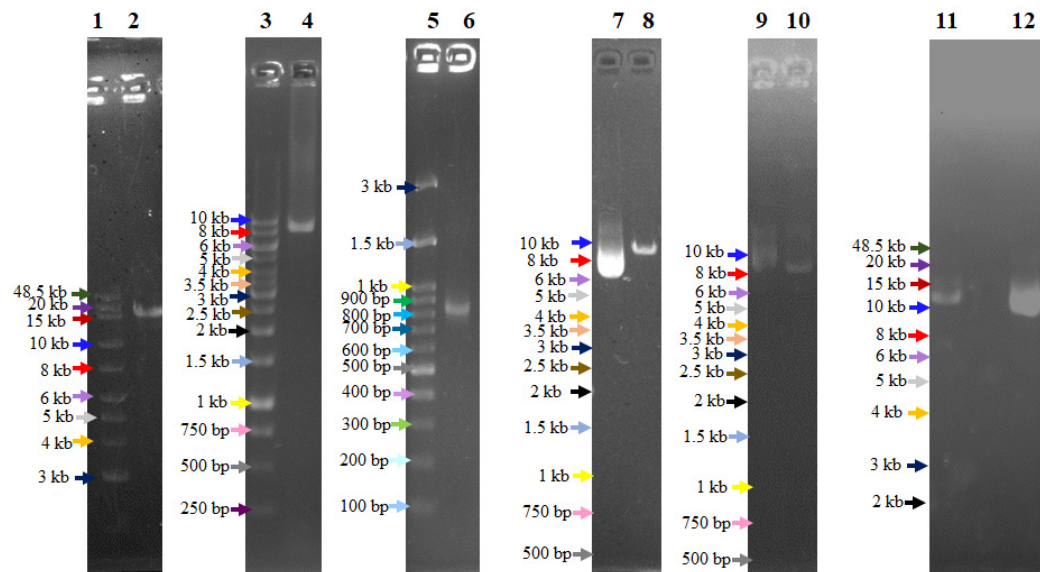
A. ZV mini-rep 1-GFP

ATCAAGCATGGGATATAAAGAGTTCAGAAGATCTGGCTGCCATGCTGAGAATAATCAATGCTAGGAAGGAGAAGAAGA
GACGAGGCGCAGATACTAGTGTCCGAATTGTTGGCCTCCTGCTGACCACAGCTATGGCAGCGGAGGTCACTAGACGTG
GGAGTGCAGGTACC^{KpnI}GGCGCGCC^{AscI}ACCGGT^{Agal}ACGCGT^{MuI}GAGGGCAGGGGAAGTCTTCTAACATGCGGGGACGTGGAGGAA^{T2A}
AATCCCGGGCCC^{T2A}ATGGTGAGCAAGGGCGAGGAGCTGTTACCGGGGTGGTGCCATCCTGGTCGAGCTGGACGGCGA
CGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCCTGAAGTTCA
TCTGCACCACCGGCAAGCTGCCCCTGCCCTGGCCACCCCTCGTGACCACCCCTGACCTACGGCGTGCAAGTTCAGCC
GCTACCCCGACCATGAAGCAGCAGCACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCT
TCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAG
CTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCTGGGGCACAAGCTGGGGTACAACCTACAACAGCCACAACGT
CTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACCTTCAAGATCCGCCACAACATCGAGGACGGCAGCGT
GCAGCTCGCCGACCACTACCAGCAGAACACCCCATCGGGCAGCGCCCGTGCTGCTGCCCGACAACCACTACCTGA
GCACCCAGTCCGCCCTGTGCATAGACCCCAATGAGAAGCGCGATCACATGGTCTGCTGGAGTTCGTGACCGCCGCCG
GGATCACTCTCGGCATGGACGAGCTGTACAAGAACTTTTGATTGCTCAAGTTGGCAGGAGACGTTGAGTCCAACCCCG^{F2A}
GGCCC^{F2A}GGCGCGCC^{NotI}ATCCCGAGGAAGTGCAAAGCTGAGATGGTTGGTGGGAGCGGGGATACCTGCAGCCCTATGGGA
AAGGGTCA

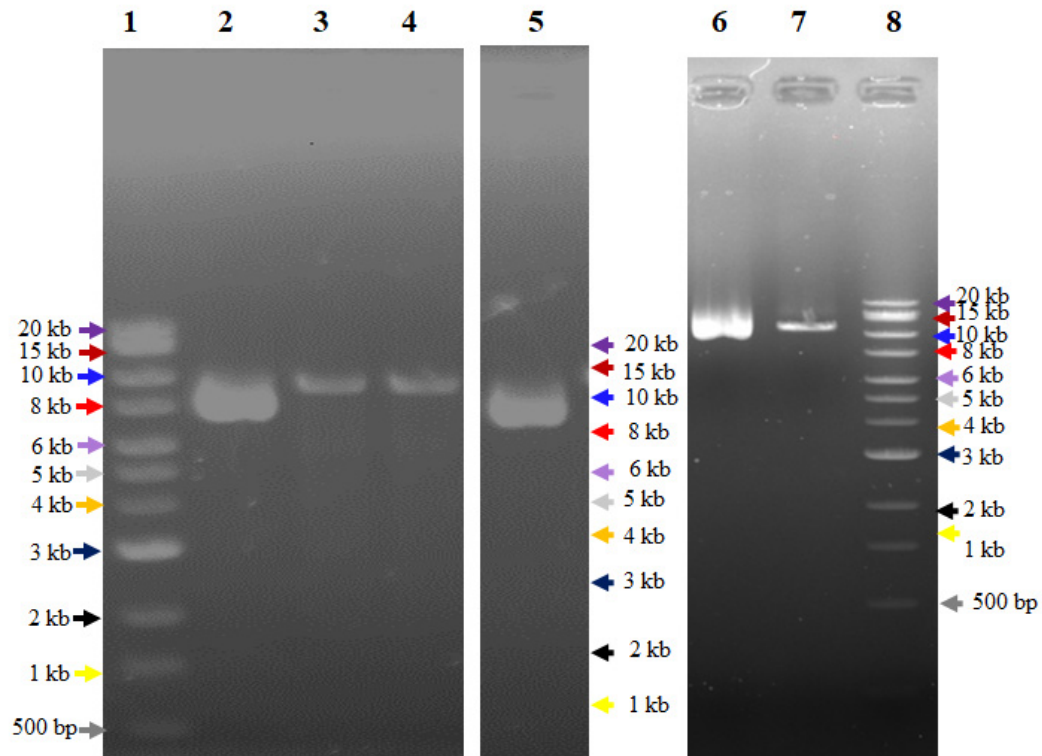
B. ZV mini-rep 1-no reporter

CATGGGTATATCAAGATGTTCAAGAAGATCTGGCTGCCATGCTGAGAATAATCAATGCTAGGAAGGAGAAGAA
GAGACGAGGCGCAGATACTAGTGTCCGAATTGTTGGCCTCCTGCTGACCACAGCTATGGCAGCGGAGGTAC
TAGACGTGGGAGTGCAGGTACC^{KpnI}GGCGCGCC^{AscI}ACCGGT^{Agal}ACGCGT^{T2A}GAGGGCAGGGGAAGTCTTCTAACATGCG
GGGACGTGGAGGAAAATCCCGGGCCC^{T2A}ATGGTGAGCAAGGGCGAGGAGCTGTTACCGGT^{MuI}ATCGAT^{ClaI}GATATCA^{EcoRV}
CTCTCGGCATGGACGAGCTGTACAAGAACTTTGATTGCTCAAGTTGGCAGGAGACGTTGAGTCCAACCCCG^{F2A}
GGCCC^{F2A}GGCGCGCC^{NotI}ATCCCGAGGAAGTGCAAAGCTGAGATGGTTGGTGGGAGCGGGGATACCTGCAGCCCTATG
GAAAGGTCAATTGATCTTGGATGTGGCAGAGGGGGCTGGAGTTACTACGCCGCCACCATCCGCAAAGTTCAAG
AAGTGAAAAGGATACAAAAAGGAGGCCCTGGTCATGAAGAACCCGTGTTGGTGCAAAGCTATGGGTGGAAC
ATAGTCCGTCTTAAGAGTGGGGTGGACGTCTTTCATATGGCGGCTGAGCCGTGTGACACGTTGCTGTGTGACA
TAGGTGAGTCATCATCTAGTCTGAAGTGGAAGAAGCACGGACGCTCAGAGTCTCTCCATGGTGGGGGATT
GGCTTGAAAAAAGACCAGGAGCCTTTTGTATAAAAGTGTGTGCCATACACCAGCACTATGATGGAAACCCT
GGAGCGACTGCAGCGTAGTATGGGGGAGGACTGGTCAGAG

Supplemental Figure 1. The nucleotide sequence analysis of two constructs: ZV mini-rep 1-GFP (A) and ZV mini-rep 1-no reporter (B). The figure highlights the presence of restriction enzyme sites and includes the sequences of T2A and F2A peptides, which have been marked for reference.



Supplemental Figure 2. Gel electrophoresis analysis of ZIKV mini-replicons containing the GFP reporter. The sizes of the ZIKV Natal RGN replicon, mini-replicons, and cDNA fragments F1 to F4 were analyzed using agarose gel electrophoresis. The gel lanes are labeled as follows: 1, DNA marker (1 kb Extend DNA ladder); 2, ZIKV replicon (15058 bp); 3, DNA marker (1 kb DNA ladder); 4, Fragment 1 (8326 bp); 5, DNA marker (100 bp DNA ladder); 6, Fragment 2 (853 bp); 7, ZIKV mini-rep 1-GFP (8726 bp); 8, ZIKV mini-rep 1-GFP cut by NotI; 9, ZIKV mini-rep 2-GFP (8890 bp); 10, ZIKV mini-rep 2-GFP cut by NotI; 11, ZIKV mini-rep 3-GFP (9868 bp); 12, ZIKV mini-rep 3-GFP cut by NotI.



Supplemental Figure 3. Gel electrophoresis analysis of ZIKV mini-replicons containing the CYP reporter (cyan fluorescent protein-linker-yellow fluorescent protein). The sizes of the mini-replicons and cDNA fragment F5 were determined through agarose gel electrophoresis. The gel lanes are labeled as follows: 1, DNA marker (1 kb Extend DNA ladder); 2, Fragment 5; 3, ZIKV mini-rep 2-CYP (9730 bp); 4, ZIKV mini-rep 2-CYP cut by NotI; 5, Fragment 5; 6, ZIKV mini-rep 3-CYP (10708 bp); 7, ZIKV mini-rep 3-CYP cut by NotI; 8, DNA marker (1 kb Extend DNA ladder).