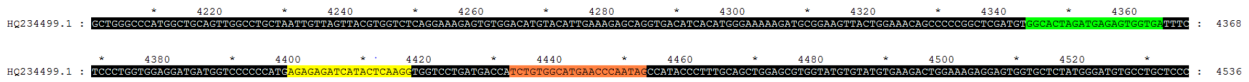
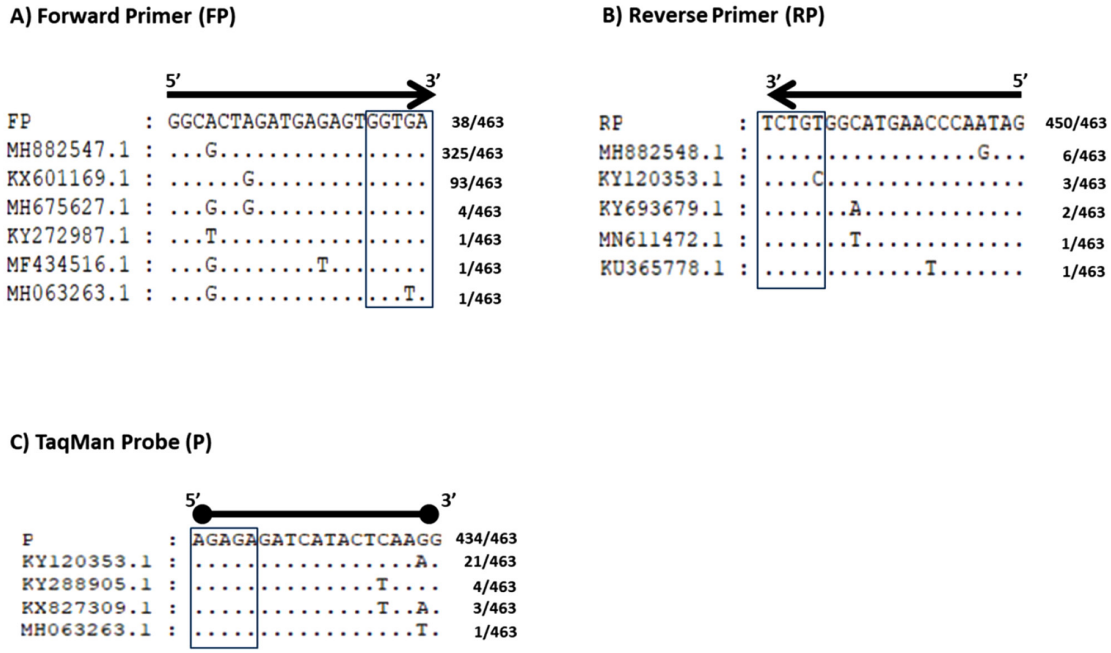
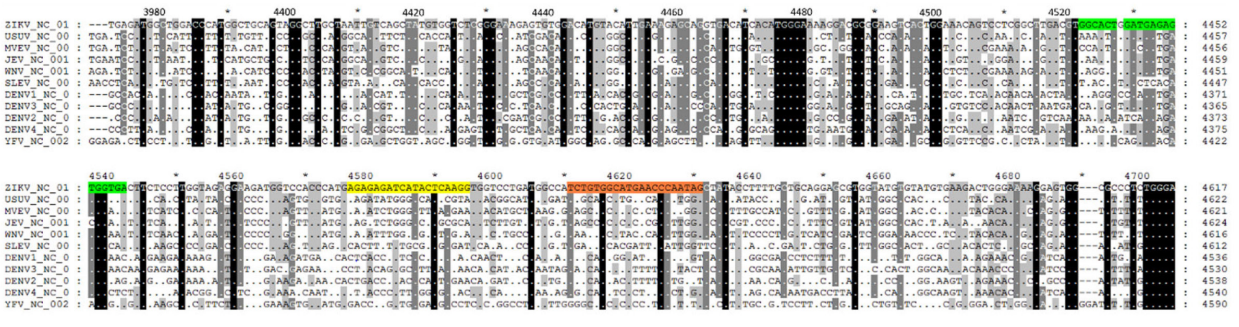
Supplementary Figure S1. Nucleotide positions of the primers-probe set on the ZIKV gene sequence (GenBank accession number: HQ234499). Green indicates forward primer, yellow indicates TaqMan probe, and orange indicates reverse primer.



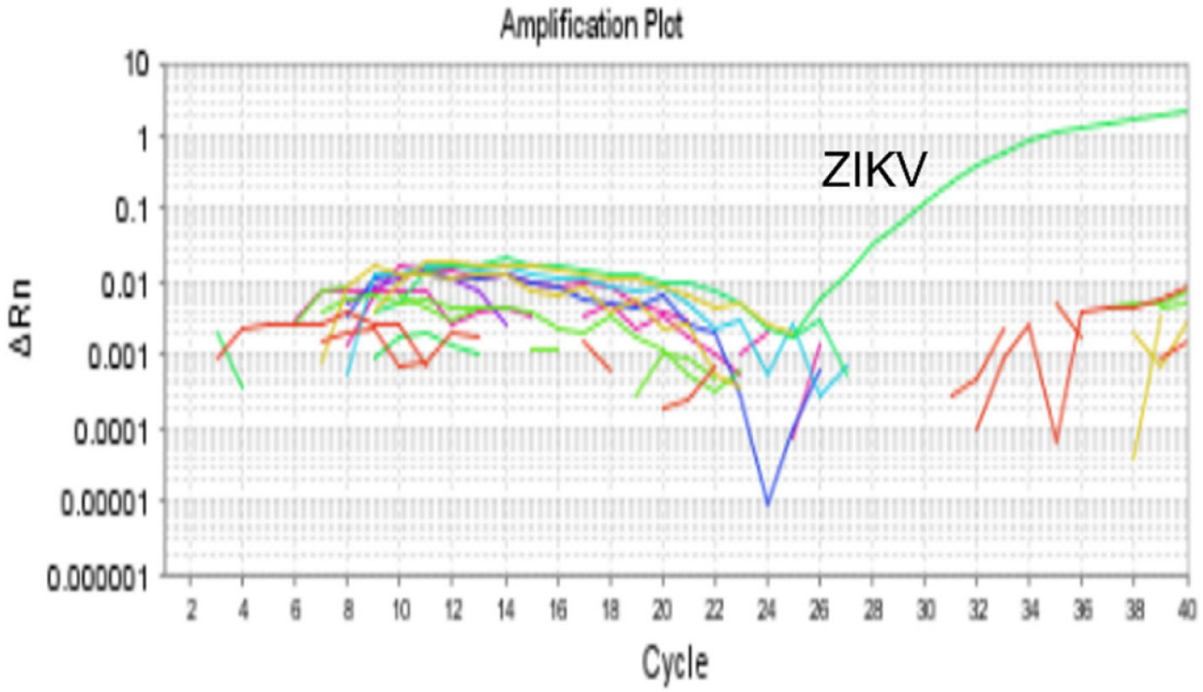
Supplementary Figure S2. Nucleotide mismatches of the qRT-PCR primers and probe against 463 ZIKV genome sequences. The top sequences are primer (FP and RP) and probe (P) sequences. Total numbers of ZIKV genomes with 100% sequence similarity to the primers and probe are shown at the end of the top sequences. Only the ZIKV genomes with nucleotide mismatches to the primers and probe are listed. The dots indicate the same nucleotides as the top sequence. The boxes indicate the nucleotide positions which are critical for priming and amplification.



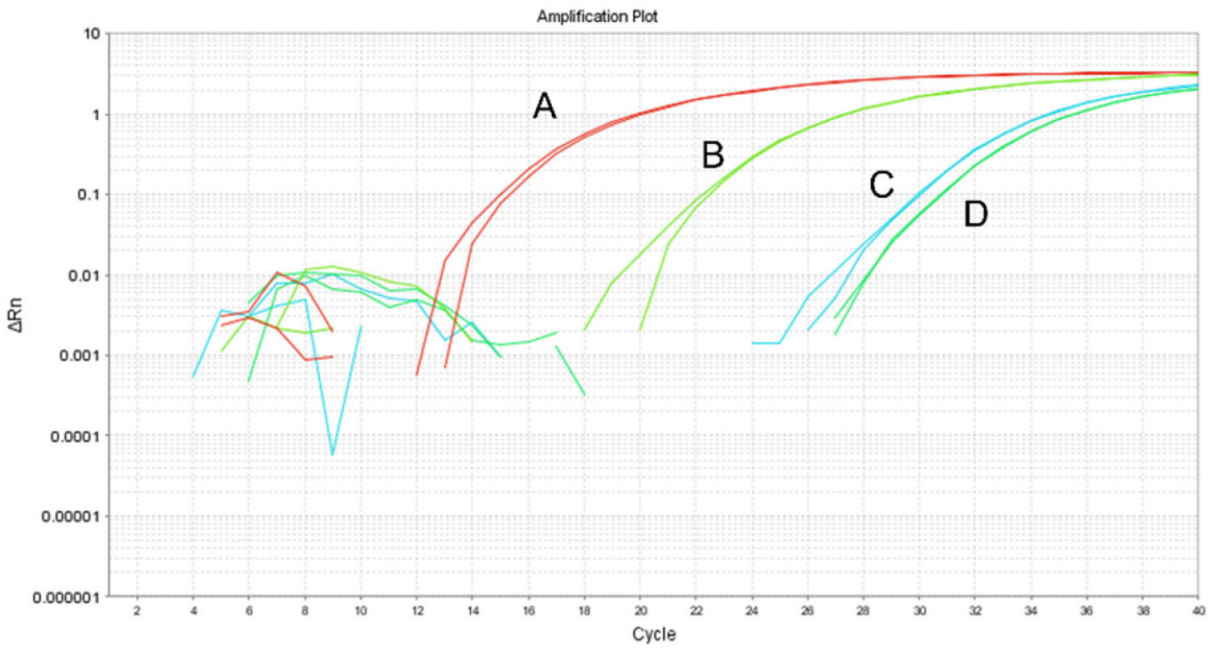
Supplementary Figure S3. *In silico* cross reactivity analysis of primers-probe sets for ZIKV detection using GeneDoc. Green indicates forward primer; yellow indicates probe and orange indicates reverse primer. Dots indicate same nucleotide with the reference ZIKV strain on top of the alignment.



Supplementary Figure S4. Cross-reactivity of the ZIKV qRT-PCR assay. Amplification plots of different arboviruses including ZIKV, DENV-1, DENV-2, DENV-3, DENV-4, CHIKV, JEV, LGTV, GETV, and SINV. No amplification was observed against other arboviruses.



Supplementary Figure S5. Coverage of the ZIKV qRT-PCR assay. Amplification plots of all ZIKV strains used in this study. A) ZIKV P6-740, B) ZIKV PRVA5C59 C) ZIKV H/FP/2013 and D) ZIKV MR766.



Supplementary Table S1. Characteristic of the designed primers-probe by the IDT's OligoAnalyzer

Name	Sequence 5'-3'	Binding site	Length of nucleotide (bp)	Tm (°C)	GC %	Self-dimer (kcal/mol)	Hetero-dimer (kcal/mol)	Hairpin (Tm, °C)	Position (bp)	Size (bp)
FP	GGCACTAG ATGAGAGT GGTGA		21	56.0	52.4	-4.89	-8.16 -5.13	38.4 36.0 33.7	4344-4364	109
RP	CTATTGGG TTCATGCC ACAGA	TCTGTGGC ATGAACCC AATAG	21	54.9	47.6	-5.38	-8.16 -3.90	30.0 28.1 25.7 20.1	4433-4453	
P	AGAGAGAT CATACTCA AGG		19	47.5	42.1	-4.62	-5.13 -3.90	36.6 40.1 33.7	4398-4417	

FP, forward primer; RP, reverse primer; P, TaqMan probe.

Supplementary Table S2. Comparison of Ct values between Genesig Real-Time qRT-PCR ZIKV Detection Kit and the designed qRT-PCR assay

No	Sample (PFU/ml)	CT Mean (Genesig Real-Time qRT-PCR ZIKV Detection Kit)	CT Mean (Designed qRT-PCR assay)
1	Ser_	23.07	23.32
2	Ser_	26.73	26.35
3	Ser_	29.89	29.65
4	Ser_	36.08	34.54
5	Ser_	35.57	34.24
6	Ser_Mock	–	–
7	Sal_	23.65	24.01
8	Sal_	27.21	26.88
9	Sal_	30.78	29.98
10	Sal_	35.25	33.62
11	Sal_	35.45	34.68
12	Sal_Mock	–	–
13	Uri_	23.45	23.08
14	Uri_	27.06	25.95
15	Uri_	30.37	29.06
16	Uri_	33.10	32.14
17	Uri_	36.43	35.96
18	Uri_Mock	–	–

Ser, serum; Sal, saliva; Uri, urine.