

TABLE I
Conserved regions in flavivirus genomes

Protein	CR	Conserved region (amino acid)	Identical sites (%)	Pairwise % Identity	Conserved region (nucleotide 5'-3')
Env	CRE_1	DRGWG(N/K)(G/H)(C/G)(E)(L/F)(F/S)GK(G/R)	57,1%	98,60%	GAYMGNGGNTGGGGNAABSRNTGYGRNYTNTYYGG- NAARRGN
	CRE_2	AWDF(G/S/R/S)	83,3%	99,40%	GCNTGGGAYTTYVGNTCN
	CRNS1_1	WC(C/S)(R/K)(S/N/E/T/A/C)	50,0%	95,50%	TGGTGYTSMRNDVNTGY
	CRNS1_2	CWY(G/A/R/P/S)(M/V)E(I/V)RP	66,7%	97,40%	TGYTGGTAYBSNRTGGARRTHMGNCCN
	CRNS3_1	G(T/S)(S/F)GSP(I/T)	57,1%	99,90%	GGNWSNTYNGGNWSNCCNAYH
	CRNS3_2	(G/S)(L/C)(Y/L/C)GNG	50,0%	99,90%	RGN YKNTDBGGNAAYGGN
	CRNS3_3	L(A/V)P(T/S)R(V/A)(V/G)	42,9%	99,90%	YTNGYNCCNASNMGNGYNGKNBYN
NS3	CRNS3_4	(S/G/R)(I/L/T/V)AARG	66,7%	96,50%	RGBDYNGCNGCNGMNGGN
	CRNS3_5	MTAT(P/S)(P/R)G	71,4%	99,96%	ATGACNGCNACNYCNCNNGGN
	CRNS3_6	(T/A)D(I/M)(S/A)(E/V)(M/V)GA(N/Y)	33,3%	99,30%	RCNGAYATKNCGWRRITGGGNGCNWAY
	CRNS3_7	AS(A/T/N)(Q/P)RRGR(I/V)(G/S)R	63,6%	95,40%	WSNGCNRMCMRMGNMNGGNMGRNTRNRMNGM
	CRNS5_1	DLGCG(R/C)(G/S)W(S/C/N)(Y/C)	66,7%	98,20%	GAYYTNGGNTGYGGNHGNGGNRGNITGGWVNTAYTRY
	CRNS5_2	(C/Y/G)D(I/V)GES	66,7%	99,90%	KRYGAYRTHGGNGARWSN
	CRNS5_3	P(L/F)S(C/R/K)NS(T/N)(H/D)EMY	54,5%	96,00%	CCNYTNTSNMRNAA YTCNAMNSAYGARATGTAY
NS5	CRNS5_4	AM(T/N)DT(T/K)	66,7%	99,95%	GCNATGAMNGAYACNAMN
	CRNS5_5	GQQRVFK(E/D)KVDI	91,7%	99,60%	GGNCARCARMNGNTTYAARGANAARGTNGAYACN
	CRNS5_6	(C/G/R)(V/A/I)(Y/H)(N/I/H)(M/T)MG(K/M)REKK	50,0%	96,70%	BGYRYNYAYMWYAYRATGGGNWRMNGARARAAR
	CRNS5_7	EFG(K/R/V)AKGSR(A/V)IW(Y/F)MWLG(A/S) R(F/Y)L(E/D)F(E/K)ALG(F/S)(L/M)N(E/L)D(H/R)W	67,6%	95,20%	GARTTYGGNRDRGCNAARGGNAGYMGNGYNATHITGGT- WYATGGYTNGGVRSNMGNNTWYYTNGADTTYRARGC- NYTNGGNTYHTNAAYDWRGAYCRYTGG
	CRNS5_8	(Y/F)ADDTAGWDI	90,0%	99,90%	TWYGCNGAYGAYACNGCHGGNTGGGAYACN
	CRNS5_9	(D/E/A/N)Q(R/I)(G/R)(S/T/I)(G/E/V)(Q/R)V(V/G)TY	36,4%	95,30%	RMHCARMKDSGNWBNGDNCRRRTNGKKNACNTAY
	CRNS5_10	L(N/G)I(F/I/Y/L)TN	66,7%	96,00%	YTNRRRYACNHWACNAAY
NS1	CRNS5_11	(S/T/N)GDD(C/S)(V/L/E/A)(V/G)(K/R/E)P	44,4%	97,30%	AVYGGNGAYGAYWGYSHNGKNV RVCCN
	CRNS5_12	(V/G/F)PF(C/G/W)(S/C)(H/N/R/P)(H/R/S)F	37,5%	95,30%	KKNCCNTTYKGBTSNMVMRYTTY
	CRNS5_13	(Y/N/D)F(H/Q/D)(R/K)(D/G/Y)(L/Q/M)(R/C)	25,0%	98,50%	DAYTTYSAHMRNRMGNKRYHWNHGN
	CRNS5_14	R(T/P)(T/S)WS(I/V)KH	57,1%	96,10%	MGNMNCWNTGGWSNRWHCAY
	CRNS5_15	W(M/I)T(T/A/N)(E/G/Q)(D/N/E)(M/R)L	37,5%	99,70%	UGGAUSACNRMNSRRRABAKGYUN
	CRNS5_16	(V/A)WN(R/K)(V/L)W(I/V)	42,9%	99,80%	GYNTGGAAYMRNKNTTGGRTN



TABLE II
Universal primes designed in this study

Protein	Name	Primer sequence (5'-3')	Primer size (nt)	T _m (°C) (average)	Degeneracy
Env	CRE_1F	TGYGRNYTNTYYGGNAARRG	20	47.6 - 69.6 (58,6)	8192
	CRE_2F	GCNTGGGAYTTYVGNTCN	18	49.1 - 62.5 (55,8)	768
	CRE_2R	NGANCBRAARTCCCANGC	18	49.3 - 63.1 (56,2)	768
NS1	CRNS1_1F	TGGTGYTSYMRNDVNTGY	18	46.6 - 68.4 (57,5)	9216
	CRNS1_1R	RCANBHNYSARCACCA	18	46.0 - 68.1 (57,0)	9216
	CRNS1_2F1	TGGTAYBSNRTGGARRTHMGNC	23	54.4 - 72.0 (63,2)	9216
	CRNS1_2R1	GGNCKDAYYTCCAYNSVRTACCA	23	53.9 - 72.0 (62,9)	9216
	CRNS1_2R2	CKDAYYTCCAYNSVRTACCA	20	47.6 - 65.6 (56,6)	2304
	CRNS1_2R3	AYYTCCAYNSVRTACCARCA	20	49.9 - 65.2 (57,5)	768
NS3	CRNS3_1F	GGNWSNTYNGGNWSNCCNAYH	21	53.2 - 72.1 (62,6)	786432
	CRNS3_1R1	TNGGNSWNCCNRANSWNCC	19	53.0 - 69.1 (61,0)	131072
	CRNS3_2F	RGNYKNTDBGNAAYGGN	18	43.8 - 70.5 (57,1)	36864
	CRNS3_3F	YTNGYNCCNASNMNGYNGKNBYN	24	52.4 - 81.3 (62,1)	25165824
	CRNS3_3R1	NRVNMCNRCNCKNSTNGG	18	46.5 - 73.4 (59,9)	393216
	CRNS3_4F	RGBDYNGCNGCNMGNGGN	18	51.7 - 77.7 (64,7)	73728
	CRNS3_5F1	ATGACNGCNACNYCNCNNGG	20	58.2 - 71.3 (64,7)	4096
	CRNS3_5R1	CCNSGNGRNGTNGCNGTCAT	20	57.1 - 72.6 (64,8)	4096
	CRNS3_6F1	GAYATNKCNGWRRRTGGGNGC	20	50.5 - 68.5 (62,1)	2048
	CRNS3_6R1	TWNGCNCCCAYYWCNGMNATRTC	23	54.2 - 71.1 (62,6)	16384
	CRNS3_6R2	GCNCCCAYYWCNGMNATRTC	20	51.7 - 68.3 (60,0)	2048
	CRNS3_7F1	NGCNRMNCMRMGNGG	18	50.3 - 77.3 (61,1)	65536
	CRNS3_7R1	CKNCCNCKNCKYKGNKYNGC	20	50.7 - 80.9 (65,8)	131072
NS5	CRNS5_1F1	TNGGNTGYGGNHGNGGNRGNTGG	23	59.0 - 80.2 (69,6)	49152
	CRNS5_1F2	TGYGGNHGNGGNRGNTGG	18	53.0 - 74.1 (63,5)	3072
	CRNS5_1F3	GAYYTNGGNTGYGGNHGNGG	20	52.8 - 73.1 (62,9)	6144
	CRNS5_1R1	CCNCDNCCRCANCCNARRTC	20	55.6 - 72.3 (63,9)	6144
	CRNS5_1R2	CCANCYNCCNCDNCCRCANCC	21	59.3 - 76.6 (67,9)	12288
	CRNS5_1R3	CCANCYNCCNCDNCCRCA	18	54.3 - 72.4 (63,3)	3072
	CRNS5_2F	KRYGAYRTHGGNGARWSN	18	42.8 - 65.7 (54,2)	12288
	CRNS5_3F1	AAATCNAMNSAYGARATGTA	20	46.8 - 58.1 (52,4)	512
	CRNS5_3R1	TNGARTTNYKNSANARNGG	19	42.3 - 65.1 (53,7)	32768
	CRNS5_3R2	TACATYTCRTSNKTNGARTT	20	45.0 - 58.3 (51,6)	512
	CRNS5_4F	GCNATGAMNGAYACNAMN	18	45.7 - 60.7 (53,2)	2048
	CRNS5_4R	NKTNGTRTCNKTCATNGC	18	42.8 - 61.2 (52,0)	2048
	CRNS5_5F1	GTNTTYAARGANAARGTNGAYAC	23	47.1 - 61.3 (54,2)	1024
	CRNS5_5F2	GTNTTYAARGANAARGTNGA	20	43.6 - 57.2 (50,4)	512
	CRNS5_5R1	TCYTTRAANACNCKYTGYTGNC	23	54.2 - 69.6 (61,9)	2048
	CRNS5_5R2	TTRAANACNCKYTGYTGNC	20	50.3 - 66.0 (58,1)	1024
	CRNS5_5R3	TTNTCYTTRAANACNCKYTGYTG	23	50.3 - 65.3 (57,8)	2048
	CRNS5_5R4	TCYTTRAANACNCKYTGYTG	20	48.1 - 63.0 (55,5)	512
	CRNS5_6F1	ATGGGNAWRMGNGARAARA	20	49.0 - 62.8 (55,9)	512
	CRNS5_6F2	AYRATGGGNAWRMGNGARA	20	47.8 - 65.2 (56,5)	1024
	CRNS5_6R1	TTYTTYTCNCKYWTNCCCAT	20	47.9 - 61.0 (54,5)	512
CRNS5_7F1	GGNTYYHTNAAAYDWRGAY- CRYTGG	24	50.0 - 70.8 (60,6)	36864	
CRNS5_7F2	TNGGNTYYHTNAAAYDWRGA	19	39.6 - 62.6 (51,1)	18432	

Protein	Name	Primer sequence (5'-3')	Primer size (nt)	Tm (°C) (average)	Degeneracy
	CRNS5_7F3	TTYRARGCNYTNGGNTYYHTNAA	23	48.0 - 69.5 (58,7)	49152
	CRNS5_7F4	TNGADTTYRARGCNYTNGG	19	44.7 - 63.6 (54,1)	3072
	CRNS5_7F5	TGTGGYTNGGVRSMGNT- WYYTNGA	25	55.7 - 75.5 (65,6)	98304
	CRNS5_7F6	GYNATHGGTWEATGTG- GYTNGG	23	51.7 - 65.5 (58,6)	768
	CRNS5_7F7	AGYMGNGYNATHGGTWEAT- GTGG	24	52.7 - 69.4 (61,0)	1536
	CRNS5_7F8	GNGYNATHGGTWEATGTGG	20	47.4 - 61.4 (54,4)	384
	CRNS5_7F9	ATHGGTWEATGTGGYTNGG	20	48.6 - 59.5 (54,0)	96
	CRNS5_7R1	CTNCCYTTNGCYHYNCCRAAYTC	23	52.5 - 70.6 (61,5)	6144
	CRNS5_7R2	CTNCCYTTNGCYHYNCCRAA	20	49.2 - 68.5 (58,8)	3072
	CRNS5_7R3	CCYTTNGCYHYNCCRAAYTC	20	48.8 - 67.2 (58,0)	1536
	CRNS5_7R4	ACCADATNRCNCKRCTNC- CYTTNGC	25	56.8 - 74.2 (65,5)	12288
	CRNS5_7R5	CCACATRWACCADATNRCNCKRCT	24	55.1 - 69.1 (62,1)	1536
	CRNS5_7R6	CCNARCCACATRWACCADAT	20	52.1 - 60.0 (56,0)	96
	CRNS5_7R7	CKNSYBCCNARCCACATRWACCA	23	56.7 - 71.4 (64,0)	3072
	CRNS5_8F1	GAYGAYACNGCHGGNTGGGAYAC	23	58.2 - 71.8 (65,0)	384
	CRNS5_8F2	GCNGAYGAYACNGCHGGNTGGGA	23	62.1 - 75.2 (68,6)	768
	CRNS5_8F3	GAYGAYACNGCHGGNTGGGA	20	56.5 - 69.4 (62,9)	192
	CRNS5_8R1	TCCCANCCDGCNGTRTCRTCNGC	23	62.6 - 75.0 (68,8)	768
	CRNS5_8R2	TCCCANCCDGCNGTRTCRTC	20	57.0 - 69.1 (63,0)	192
	CRNS5_9F1	GNWBNGDNCRRGTNGKNACNTA	22	48.6 - 73.4 (61,0)	589824
	CRNS5_10F	YTNRRYACNHWHACNAAY	18	39.7 - 61.0 (50,3)	36864
	CRNS5_11F1	GAYGAYWGYSHNGKNVRVCC	20	48.8 - 72.7 (60,7)	55296
	CRNS5_11R1	GGBYBNMCNDSRCWRTCRTCNC	23	56.6 - 77.6 (67,1)	221184
	CRNS5_12F1	CCNTTYKGBTSNMVMRYTT	20	46.1 - 69.1 (57,6)	36864
	CRNS5_12R1	AARYKRBKNSAVCMRAANGG	20	45.4 - 70.8 (58,1)	36864
	CRNS5_13F	DAYTTYSAHMRNMGNKRYHWN- HGN	24	47.9 - 76.2 (62,0)	21233664
	CRNS5_13R1	CDNWDYMNCKNYKDTSRAA	20	42.1 - 72.4 (57,2)	884736
	CRNS5_14F1	GNMCNWCNTGGWSNRWHCA	19	51.8 - 66.5 (59,1)	49152
	CRNS5_15F	UGGAUSACNRMNSRRRABAK- GYUN	24	53.4 - 72.2 (62,8)	98304
	CRNS5_15R1	CMUVUYYYSNKYNGUSAUCCA	21	48.9 - 68.1 (58,5)	12288
	CRNS5_16F1	GYNTGGAAYMRNKNTTGG	18	41.8 - 63.4 (52,6)	2048
	CRNS5_16R1	NAYCCANAMNYKRTTCCA	18	43.7 - 61.9 (52,8)	2048

TABLE III
Primer set for dengue virus (DENV) (1-4) and Zika virus (ZIKV) for use with CRNS5_7NR6 reverse primer

Virus	Primer name	Sequence (5'→3')	Length	Tm (°C)	Tm mean (°C)	Degeneracy	Hairpin	Self-dimer	Specificity (3 nt na 3' conserved; max de 5 mismatches)	Amplicon (pb)	
DENV1	DENVIF1	CCAACYATAGAR- GAAGGAA	19	48.2 - 53.4	50,8	4	41.2	9.5	DENV2 (1/217), DENV4 (170/170)	-	
	DENVIF2	AGAAAYATGG- AGGRATGC	18	45.9 - 51.3	48,6	4	N	N	DENV3 (163/163)	-	
	DENVIF3	TTYACAAT- GRCTCACAGGAA	20	52.0 - 57.3	54,6	4	N	N	DENV2 (217/217), DENV4 (158/170)	713	
	DENVIF4	ACATGGCCTAY- CATGGATCA	21	58.6 - 61.0	59,8	2	66.7	25.2	N	536	
	DENVIF5	GAAAYCAATG- GAACTCAG- CAAAAG	25	57.9 - 59.8	58,8	2	N	0.1	DENV3 (5/163)	194	
	DENVIF6	GGAACCTCAG- CAAAAGARG- CAGTGG	25	63.7 - 65.2	64,7	2	37.4	N	-	184	
	DENVIF6.1	AACTCAG- CAAAAGARG- CAGTGG	23	60.9 - 62.6	61,7	2	37.4	1.6	-	182	
	DENVIF6.2	ACTCAG- CAAAAGARG- CAGTGG	21	59.0 - 60.8	59,9	2	37.4	1.6	-	181	
	DENVIF7	GGATAYATACTYA- GAGACATATCA	24	50.2 - 54.8	52,5	4	N	20.3	N	-	
	DENVIF8	CAARTCATATG- CACARATGTG- GCAG	25	60.0 - 62.4	61,2	4	45.7	14.0	N	-	
	DENVIR1	TTTTATRTTYTC- TATCCTCTG	21	45.9 - 50.0	47,9	4	N	N	-	219	
	DENVIR2	TCRTCARTATGC- CATGTTGA	20	52.6 - 57.0	54,8	4	34.0	4.2	-	305	
	DENVIR3	GATGGYT- TRACCTCATATGA	20	49.8 - 54.6	52,2	4	40.2	11.3	N	-	
	DENV2	DENV2F1	ATAGGGGART- CRICACCAA	19	52.6 - 57.3	54,9	4	53.3	None	DENV1 (72/272), DENV3 (163/163), JEV (201/203), MVEV (1/12), SLEV (28/31), WNV (125/126)	-

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Virus	Primer name	Sequence (5'→3')	Length	T _m (°C)	T _m mean (°C)	Degeneracy	Hairpin	Self-dimer	Specificity (3 nt na 3' conserved; max de 5 mismatches)	Amplicon (pb)
	DENV2F2	CYGGAAAY- ATAGTGTCAACAG	21	52.9 - 58.2	55,5	4	41.5	None	DENV1 (264/272), DENV3 (53/163), L _{ANV} (3/3), TBEV (14/137)	766
	DENV2F3	AAYATAGTGCAT- CAGTGAAYATGA	25	53.7 - 58.5	56,1	4	35.8	12.1	DENV1 (272/272), WNV (3/126)	761
	DENV2F4	TGAGYGGAGTG- GAAGGAGAA	20	57.6 - 60.5	59,0	2	None	None	DENV1 (270/272), DENV3 (163/163), DENV4 (168/168)	-
	DENV2F5	GCWCGYAGGCT- GTTGAA	18	57.6 - 61.3	59,4	4	None	26.3	N	176
	DENV2F6	CTYCATCTT- GARGGAAAGT- GTGA	23	56.7 - 60.0	58,3	4	47.7	9.0	DENV4 (139/170)	119
	DENV2F7	GGAGTG- GAAGGAGA- AGGRC ² RCAC	24	61.9 - 67.0	64,4	4	39.5	None	DENV1 (194/272), DENV3 (149/163), DENV4 (145/170)	-
	DENV2F8	TGATYAYA- GATTYACAAATGA	21	45.4 - 53.2	49,3	8	33.7	None	DENV1 (3/272), DENV3 (163/163)	724
	DENV2F9	ACTGGATCAG- CATCWTCYAYGG	22	57.4 - 62.4	59,9	8	47.1	10.7	N	500
	DENV2F10	TTYR- CAAGAAARGT- GAGAAG	20	48.8 - 56.0	52,4	8	56.6	None	-	245
	DENV2R1	GAAAT- CATR ² TCAC ² TGAT- GACACTA	25	55.0 - 57.4	56,2	2	33.4	1.4	-	71
	DENV2R2	CTTGAAAT- CATR ² TCAC ² TGAT- GAC	24	54.1 - 56.6	55,3	2	39.0	0.8	DENV1 (263/272)	74
	DENV2R3	CATYCTTGAAT- CATR ² TCAC ² TG	23	52.0 - 56.2	54,1	4	39.0	None	DENV1 (264/272), DENV3 (162/163)	72
	DENV2R4	TCTGTCA ² TWGC- CATY ² TGTG	19	52.6 - 55.2	53,9	4	33.3	None	DENV1 (272/272), DENV3 (156/163), DENV4 (109/170)	398
	DENV2R5	TTCCAY ² TRITCT- CATCAGTGA	22	52.9 - 57.3	55,1	4	35.6	2.8	N	623
	DENV2R6	GT ² YTCACACT ² TC- CYTCAAG	20	52.6 - 55.7	54,1	4	None	None	SLEV (12/31)	710
	DENV2R7	TG ² TCCT ² TY- CATR ² GGTTG	22	54.4 - 58.8	56,6	4	36.3	12.0	N	-



Virus	Primer name	Sequence (5'→3')	Length	Tm (°C)	Tm mean (°C)	Degeneracy	Hairpin	Self-dimer	(3 nt na 3' conserved; max de 5 mismatches)	Specificity	Amplicon (pb)
DENV3	DENV2R8	TCCYTCTCCYTC-CATCTG	18	52.3 - 56.5	54,4	4	None	None	DENV1 (9/272), DENV3 (29/163), DENV4 (2/170), SLEV (2/31), WNV (22/126)	-	-
	DENV3F1	GGAGAAATCYT-CACCAAGYCCAAC	23	59.2 - 63.3	61,25	4	41.2	2.0	DENV1 (272/272), DENV4 (1/170)	-	-
	DENV3F2	AGATCYTCAC-CAAGYCCAA-CAGTG	25	61.2 - 65.0	63,1	4	None	None	DENV2 (3/217), JEV (108/203)	-	-
	DENV3F3	CGACATGTYAAT-GCRGAACCA	21	56.9 - 61.5	59,2	4	37.3	24.9	N	653	653
	DENV3F4	CATGTYAAT-GCRGAAC-CAGAAACAC	25	59.4 - 63.3	61,3	4	30.8	0.4	-	649	649
	DENV3F5	GTYAAT-GCRGAAC-CAGAAACAC	25	62.0 - 65.9	63,9	4	None	3.4	-	647	647
	DENV3F6	CRGAACACACCA	25	61.0 - 65.4	63,2	4	41.3	None	-	640	640
	DENV3F6.1	GAACACAGAAA-CACCAAYATGGA	23	58.7 - 61.3	60	2	41.3	None	DENV1 (2/272)	638	638
	DENV3F7	AYAAAAC-GTGGGCTTAC-CAYGGATC	25	61.4 - 65.7	63,5	4	75.3	33.5	N	541	541
	DENV3F8	TGGGCTTACCAY-GGATCYTATGAAG	25	60.5 - 64.0	62,2	4	46.8	15.4	N	533	533
	DENV3F9	GCYACAGGTC-CAGCCTCCTC-CATGA	25	67.7 - 70.1	68,9	2	61.5	11.5	N	503	503
	DENV3F10	TGGAGRAC-CYTGGGAAGGAA	20	58.2 - 62.1	60,1	4	52.7	22.2	N	296	296
	DENV3F11	GGRCARAGAAC-GTGAATC	19	54.0 - 58.2	56,1	4	None	8.9	DENV1 (4/272), DENV2 (2/217), DENV4 (10/170), MVEV (1/12), TBEV (1/137)	135	135
DENV3F12	CAGTGGAGTRGA-AGGRGAA	19	52.7 - 58.5	55,6	4	None	None	DENV1 (270/272), DENV2 (217/217), DENV4 (168/170), JEV (1/203), LANV (3/3), TBEV (32/137)	-	-	
DENV3F13	GGACAGGTRG-GRACCTATGG	20	54.5 - 59.5	57	4	41.8	None	DENV1 (267/272), DENV2 (83/217), DENV4 (165/170)	-	-	



Virus	Primer name	Sequence (5'→3')	Length	T _m (°C)	T _m mean (°C)	Degeneracy	Hairpin	Self-dimer	Specificity (3 nt na 3' conserved; max de 5 mismatches)	Amplicon (pb)
DENV4	DENV3R1	CTCTTATRGTTCT-GCTYTC'TTCCAC	25	57.5 - 60.9	59,2	4	None	None	-	-
	DENV3R2	GTGTTTCTG-GTTCYGCATTRA-CATG	25	59.4 - 63.3	61,3	4	None	None	-	179
	DENV3R3	TGATCCTTYT-TATTCTTTC	19	45.3 - 46.6	45,9	2	None	None	JEV (1/203), SLEV (11/31), TBEV (23/137), YFV (1/40)	217
	DENV3R4	TCATCATAGTGC-CATGTTGA	20	54.4	54,4	1	None	1.6	N	251
	DENV3R5	TTCACRACTC-CR'TTATCATG	21	51.8 - 56.1	53,9	4	48.8	None	N	341
	DENV3R6	CTCTC-CACTCC'TTRK-TTTC	21	53.6 - 58.2	55,9	4	37.9	None	-	-
DENV4	DENV3R7	GATGGCTGC-CATTGRGGTATG	21	59.1 - 61.4	60,2	2	45.3	32.9	N	-
	DENV4F1	TATGGYTGAAY-TTGGTCAA	20	52.6 - 56.7	54,6	4	None	None	DENV3 (47/163), TBEV (96/137)	-
	DENV4F2	GTCRGGAAA-CATYGTGAG	18	50.6 - 55.5	53,0	4	35.6	2.2	-	768
	DENV4F3	CACCARGA-AGGRAAATGT-GAATC	23	56.3 - 59.6	57,9	4	43.9	None	-	116
	DENV4F4	CARATGGAAGCW-GAAGGAG	19	53.6 - 55.0	56,6	4	35.4	None	-	-
	DENV4R1	CAYGATTCACATT-TYCCTTC	20	50.4 - 54.7	52,5	4	37.1	None	DENV2 (192/217)	713
ZIKV	DENV4R2	AACCTRTC-GACRCCACACTC	20	57.0 - 62.7	59,8	4	None	19.6	N	-
	ZIKVF1	TGGTGCAAAGC-TATGGGTGGAACA	24	64.9	64,9	1	None	None	MVEV (12/12), WNV (104/126), YFV (8/40), SPOV (1/1)	-
	ZIKVF2	GAGTCATRCY-AGTCCCTGAAGTG	24	58.2 - 62.7	60,4	4	None	None	JEV (1/203), SPOV (1/1)	-
ZIKVF3	CATAAARAGTGT-GTCCACCA	20	53.7 - 55.0	54,3	2	42.9	None	-	756	



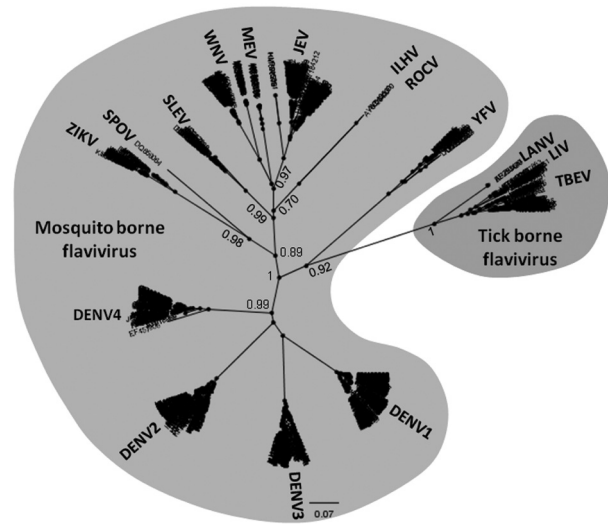
Virus	Primer name	Sequence (5' → 3')	Length	T _m (°C)	T _m mean (°C)	Degeneracy	Hairpin	Self-dimer	Specificity (3 nt na 3' conserved; max de 5 mismatches)	Amplicon (pb)
	ZIKVVF4	AAGCTGYGCT-GARGCTCC	18	57.6 - 62.8	60,2	4	50.3	18.3	N	642
	ZIKVVF5	GTGACTGGAGTY-ACAGGAA	19	53.7 - 56.3	55	2	60.1	27.6	N	437
	ZIKVVF6	AAAARGTGGACACYAGGGTGC-CAGA	25	64.2 - 68.2	66,2	4	53.6	23.4	N	370
	ZIKVVF7	AGGGTGC-CAGAYCCYCAAGA	20	60.3 - 65.3	62,8	4	71.8	6.7	WNV (13/126)	356
	ZIKVVF7.1	AGGGTGC-CAGAYCCYCAAG	19	58.7 - 63.9	61,3	4	71.8	6.7	N	356
	ZIKVVF8	GCAATATTT-GAAGAG-GAAAAAGA	23	53.6	53,6	1	None	None	-	209
	ZIKVVF9	TTGAAGAG-GAAAAAGARTG-GAAGAC	25	57.7 - 59.2	58,45	2	None	None	YFV (35/40)	202
	ZIKVVF10	AGAGARCAY-CACCTGAGAG-GAGAG	24	60.9 - 64.8	62,85	4	41.3	None	MVEV (1/12), SLEV (26/31), SPOV (1/1)	125
	ZIKVVF11	AAGGYCTYA-GACCAGTGAA	21	56.6 - 60.8	58,7	4	61.3	17.4	N	-
	ZIKVVF12	TCCATTGGTYC-CYTGCCGCCAC	24	68.0 - 71.2	69,6	4	39.2	3.2	N	-
	ZIKVVF13	CCRGGGCGRG-GATGGAGCA	19	64.0 - 69.2	66,6	4	36.1	None	N	-
	ZIKVR1	ATGTTCCACCCATAGCTTT-GCACCA	25	65.0	65	1	None	None	TBEV (1/137), WNV (23/126)	-
	ZIKVR2	AGGAGCTGRC-TYGTGTGGACACAC	25	66.6 - 70.3	68,4	4	58.1	5.7	N	80
	ZIKVR3	TCYTCATAY-TTCACTGGCCCTCC	22	57.8 - 61.2	59,5	4	None	3.3	-	125
	ZIKVR4	TCAGRCAGCT-TRCCACAGC	20	60.6 - 66.6	63,6	4	83.0	11.5	N	173
	ZIKVR5	GTCATRGC-TATTCCCTG-TRACTCCA	24	57.6 - 62.2	59,9	4	45.2	12.1	N	395

SUPPLEMENTARY DATA



Virus	Primer name	Sequence (5'→3')	Length	Tm (°C)	Tm mean (°C)	Degeneracy	Hairpin	Self-dimer	Specificity (3 nt na 3' conserved; max de 5 mismatches)	Amplicon (pb)
	ZIKVR6	CCCTTCTTGRGGRT-CTGGCACC	21	60.4 - 65.0	62,7	4	49.8	None	N	470
	ZIKVR7	GTCTTCCAY-TCTTTTTCTCTTC	23	55.5 - 57.1	56,3	2	None	None	MVEV (12/12), YFV (37/40)	626
	ZIKVR8	GGTGGCGGCARG-GRACCACAATGGA	25	69.7 - 72.7	71,2	4	63.0	12.0	N	-
	ZIKVR9	CARG-CAGTCTCCC-GRATGC	19	58.9 - 62.7	60,8	4	55.3	7.3	N	-

N: not tested.



Phylogenetic reconstruction of Flavivirus subgroups using 800-806 bp sequences of the NS5 variable region. Phylogenetic relationships were inferred using MEGA7 software (Kumar et al. 2016), adopting the Neighbor-Joining method (Saitou & Nei 1987) and the Tajima-Nei model (Tajima & Nei 1984). The rate variation among the sites was predicted with gamma distribution (shape parameter = 1). The analysis was performed from an alignment of 1,442 nucleotide sequences of 800 nt in length, corresponding to the variable region of the NS5 protein. All positions containing gaps and missing data were disregarded. The numbers correspond to the bootstrap values and branch lengths for the number of base substitutions per site. All calculations were conducted using the MEGA7 software. The rate variation among sites was predicted with a gamma distribution (shape parameter = 1).