

Supplementary Material S1

Table S1: Table with PCR assay, corresponding primers, sequences data, and genome position.

Primer Name	Sequence (5' – 3')	Target gene	Genome position	Ref.
Flavivirus First round RT-PCR				
FU1	TACAACATGGGAAAGAGAGAGAA	NS5	9043-9300	(20, 19)
9317	BBGTGATKCKHGTGTCC	NS5		
	TCGTGATGCGTGTGTCC			
Flavivirus Nested, real time PCR				
FS778	AARGGHAGYMCDGCHATHHTGGT	NS5	9091-9280	(19)
CFD2	GTGTCCCAGCCGGCGGTGTCATCA	NS5		
	GC			
Flavivirus Heminested PCR				
MAMD	AACATGATGGGRAARAGRGARAA	NS5	9043	(19)
Flavi-1	AATGTACGCTGATGACACAGCTG	NS5		(1)
	GCTGGGACAC			
Flavi-2	TCCAGACCTTCAGCATGTCTTCTG	NS5		(1)
	TTGTCATCCA			

Table S2: P-distance matrix showing the percentage (%) nucleotide similarities between mosquito *Flavivirus* detected in mosquito homogenate pools in the small fragment of the NS5. The highlighted sequences are the sequences produced in this study.

	1	2	3	4	5	6	7	8	9	10	11	12
1 Kamiti River virus (NC 005064.1)												
2 Nakiwogo virus (GQ165809.2)	67.9											
3 Lammi virus (FJ606789.2)	53.6	44.6										
4 Palm Creek virus (NC033694.1)	62.5	69.6	41.8									
5 Nhumirim virus (KJ210048.1)	50.3	47.3	46.1	47.3								
6 Calbertado virus (KX669687.1)	71.3	62.1	59.6	66.2	45.7							
7 Chaoyang virus (NC 017086.1)	45.1	50.2	71.0	48.4	48.8	49.4						
8 Barkedji virus (KC496020.1)	37.0	32.9	46.4	35.1	74.3	39.4	44.4					
9 Quang Binh virus (FJ644291.1)	71.9	67.3	54.5	70.8	50.1	77.4	50.8	35.5				
10 Culex flavivirus (JX897904.1)	65.7	75.1	47.5	70.3	35.2	70.5	58.5	20.7	73.4			
11 Culex flavivirus (AB262759.2)	60.9	74.1	44.9	70.0	32.9	71.6	51.2	13.1	69.3	93.0		
12 Aedes flavivirus (KC181923)	70.1	63.3	37.7	64.1	36.4	66.6	43.0	23.6	67.3	59.8	63.1	
13 Aedes flavivirus (MK241496.1)	69.8	71.0	50.6	71.0	43.9	61.7	49.8	38.8	65.7	68.1	63.7	68.3
14 Aedes flavivirus Narita-21 (NC 012932.1)	72.3	66.8	36.6	64.1	38.3	67.7	41.2	25.8	69.6	59.5	62.8	97.7
15 Anopheles flavivirus (KX148546.1)	60.8	70.6	46.3	72.2	44.2	65.8	52.3	33.2	67.0	69.1	70.1	59.2

16	Mosquito flavivirus (KY995162.1)	62.3	74.4	57.2	73.3	41.5	69.0	52.5	32.1	74.5	76.6	75.5	61.7
17	Mosquito flavivirus (HQ676625.1)	71.0	66.9	50.4	70.0	38.3	65.0	49.1	33.3	68.0	71.7	68.1	72.1
18	Mosquito flavivirus (HQ676624.1)	71.0	66.9	50.4	70.0	38.3	65.0	49.1	33.3	68.0	71.7	68.1	72.1
19	Culiseta flavivirus (KT599442.1)	71.7	68.3	53.1	71.7	37.8	73.1	48.5	28.3	74.1	74.9	69.6	70.3
20	KNP17MP30 An. coustani	60.8	65.5	45.2	67.2	34.3	70.7	47.6	26.6	67.3	77.2	76.2	61.0
21	KNP17MP29 An. coustani	54.1	61.5	36.5	58.4	24.7	62.3	46.2	16.5	59.9	73.8	72.8	54.1
22	KNP17MP667 An. squamosus	59.6	62.0	42.6	76.5	28.5	66.9	42.0	29.2	69.0	65.9	60.9	60.0
23	KNP17MP71 An. squamosus	45.2	52.1	33.6	43.8	15.6	49.3	39.3	6.3	52.5	68.0	73.6	51.3
24	KNP17MP666 An. squamosus	57.4	64.1	41.7	73.6	30.0	64.8	37.6	29.7	69.0	65.9	60.9	60.2
25	KNP17MP673 An. maculipalpis	61.6	67.0	51.6	71.4	43.0	64.7	57.7	32.1	70.3	69.1	64.8	63.2
26	COET17MP500 Ae. aegypti	66.2	63.1	43.6	66.4	30.8	61.1	42.4	25.2	64.3	68.1	64.2	67.4
27	COET17MP502 Ae. aegypti	71.0	66.9	50.4	70.0	38.3	65.0	49.1	33.3	68.0	71.7	68.1	72.1
28	KZN17MP145 Ae. aegypti	70.0	72.0	47.7	70.0	43.9	60.6	47.0	38.8	65.8	70.3	66.0	68.3
29	ROOD18MP126 Ae. sudanensis	64.1	65.9	48.6	62.5	39.6	56.5	44.5	37.7	59.7	62.6	57.9	60.2
30	KNP17MP653 Ae. vexans gr	57.4	64.1	41.7	73.6	30.0	64.8	37.6	29.7	69.0	65.9	60.9	60.2
31	SHI17MP544 Aedes spp.	69.8	71.0	50.6	71.0	43.9	61.7	49.8	38.8	65.7	68.1	63.7	68.3
32	KNP17MP639 Aedes spp.	62.9	66.8	52.9	71.5	54.0	70.2	54.4	35.0	79.4	68.5	65.8	67.8
		8	9	10	11	12	13	14	15	16	17	18	19
13	Aedes flavivirus (MK241496.1)												
14	Aedes flavivirus Narita-21 (NC 012932.1)						68.5						
15	Anopheles flavivirus (KX148546.1)						62.6	58.1					
16	Mosquito flavivirus (KY995162.1)						68.7	62.8	73.0				
17	Mosquito flavivirus (HQ676625.1)						91.3	72.1	62.7	68.8			
18	Mosquito flavivirus (HQ676624.1)						91.3	72.1	62.7	68.8	100.0		
19	Culiseta flavivirus (KT599442.1)						70.7	67.9	64.2	66.9	72.0	72.0	
20	KNP17MP30 An. coustani						62.9	60.8	67.2	68.9	74.6	74.6	73.7
21	KNP17MP29 An. coustani						52.0	53.8	59.8	61.3	65.3	65.3	66.8
22	KNP17MP667 An. squamosus						72.3	61.3	71.9	59.3	79.3	79.3	65.0
23	KNP17MP71 An. squamosus						44.8	50.9	40.3	52.0	51.8	51.8	52.2
24	KNP17MP666 An. squamosus						71.4	61.6	68.9	59.3	78.5	78.5	61.6
25	KNP17MP673 An. maculipalpis						66.0	63.3	89.6	69.7	69.5	69.5	62.0
26	COET17MP500 Ae. aegypti						87.8	67.3	58.6	65.1	96.9	96.9	68.5
27	COET17MP502 Ae. aegypti						91.3	72.1	62.7	68.8	100.0	100.0	72.0
28	KZN17MP145 Ae. aegypti						98.5	68.5	61.5	70.8	91.3	91.3	69.7
29	ROOD18MP126 Ae. sudanensis						93.7	60.5	53.0	61.7	84.9	84.9	64.9
30	KNP17MP653 Ae. vexans gr						71.4	61.6	68.9	59.3	78.5	78.5	61.6
31	SHI17MP544 Aedes spp.						100.0	68.5	62.6	68.7	91.3	91.3	70.7
32	KNP17MP639						72.4	68.9	67.9	70.2	69.3	69.3	72.2

Table S3: P-distance matrix showing the percentage (%) nucleotide similarities between Cell fusing agent virus detected in mosquito homogenate pools. The highlighted sequences are the sequences produced in this study.

	1	2	3	4	5	6	7
1 KJ741267.1 CFAV Galveston							
2 LR694075.1 CFAV Cairns	97.03%						
3 HM579916.1 CFAV Colima	96.21%	95.99%					
4 KP792624.1 CFAV Kenya	95.81%	96.01%	94.55%				
5 COET17MP503 Ae. aegypti	94.58%	95.58%	93.70%	95.61%			
6 COET17MP501 Ae. aegypti	93.55%	94.55%	93.08%	95.40%	97.22%		
7 GAU16MP01 Ae. caballus	93.97%	94.97%	93.51%	95.41%	97.42%	98.03%	
8 SHI17MP525 Ae. aegypti	93.54%	94.55%	92.65%	95.00%	98.02%	95.79%	96.61%

Table S4: P-distance matrix showing the percentage (%) nucleotide similarities between mosquito *Flavivirus* detected in mosquito homogenate pools in the large fragment of the NS5. The highlighted sequence is the sequence produced in this study.

	1	2	3	4	5
1 Aedes flavivirus (KC181923)					
2 Aedes flavivirus Narita-21 (NC 012932.1)	98.24%				
3 Mosquito flavivirus (KM088035.1)	40.12%	40.65%			
4 Mosquito flavivirus (KM088039.1)	40.08%	40.62%	99.73%		
5 Haslams Creek (MF352617.1)	38.84%	39.95%	72.89%	73.28%	
6 KNP17MP71 An. squamosus	37.57%	38.11%	96.58%	96.86%	73.02%

Supplementary Material S2: Sequences produced in this study and not deposited at NCBI GenBank.

>KNP17MP30 An. coustani

TGGCTGGGAAGTCGATTCTCGAGTACGAAGCACTAGGATTCCTGAATG
CGGACCATTGGGTAGCTAGAATGAATTTACCTGTGGGGTCGGAGGAGTTGGG
GTGAACTACTTTGGTAATTATCTCAAGGAGAT

>KNP17MP29 An. coustani

TGGCTAGGAAGTCCGATTCTCGAGTACGAAGTACTAGGATTCCTGAATG
CGGACCATTGGGTAGCTAGAATGAATTGAACCTGCGGGGTCGGAGGAGTTGGG
GTGAACTACTTTGGTAATTATCTCAAGGAGAT

>KNP17MP667 An. squamosus

TGGTTAGGAAGTCGATATCTAGAGTATGAGGCTTTAGGTTTCCTCAACC
AAGACCATTGGGTTTCACGTGACCATCTGCCATGTGGAGTGGGAGGCGTTGGT
GTGAACTATTTGGTAATTATCTGAAGGAAAT

>KNP17MP71 An. squamosus

TGGCTCGGAAGTCGGTTTTTGGAGTACGAAGCACTTGGGTTTCCTAAACG
AGGATCACGGTTTAACCAGCAAAAATATTGCCGACAGGGTTGCCAGATCTCGC
TTGCATTACTTTGGATACTATCTCCAGGAGAT

>KNP17MP666 An. squamosus

TGGTTAGGAAGTCGATATCTAGAGTATGAGGCTTTGGGTTTTCTCAACC
AAGACCATTGGGTTTCACGTGACCATCTGCCATGCGGAGTGGGAGGCGTTGGT
GTGAACTATTTTGGTAATTATCTGAAGGAAAT

>KNP17MP673 *An. maculipalpis*

TGGCTAGGGAGTAGATTCTTGGAATATGAAGCCCTAGGATTCTTAAACC
AAGACCATTGGGTGTCACGTGACCATTTGCCATGTGGGGTTCGGAGGCGTTGGT
GTCAATTACTTCGGAAATTACCTGAAGGAAAT

>COET17MP500 *Ae. aegypti*

TGGCTGGGAAGTCGATATCTGGAATATGAGCCCTTGGGCTTCCTCACCG
AAGACCATTGGGTAGCCCGTGAAAATTTCCCGAATGGCGTGGGAGGTCTCGGT
GTGAACTACTTTGGTAATTATCTCAAGGAGAT

>COET17MP502 *Ae. aegypti*

TGGCTGGGAAGTCGATATCTGGAATATGAGGCCTTGGGCTTCCTCAACG
AAGACCATTGGGTAGCCCGTGAAAATTTCCCGGGTGGCGTGGGAGGTCTCGGT
GTGAACTACTTTGGTAATTATCTCAAGGAGAT

>KZN17MP145 *Ae. aegypti*

TGGCTGGGAAGTCGATATCTGGAATATGAGGCCTTGGGCTTCCTCAACG
AAGACCATTGGGTAGCCCGTGAAAATTTCCCGGGCGGCGTGGGAGGTCTCGGC
GTGAACTACTTTGGCTATTACTTGTGAGAAAT

>ROOD18MP126 *Ae. sudanensis*

TGGCTGGGAAGTCGATATCTTGGATATGAGGCCTTGGGCTTCCTCAACG
AAGACCATTGGGTAGCCCGTGAAAATTTCCCGGGCGGCGTGGGAGGTCTCGTA
CTATACTACTTTGGCTATTACTTGTGATAAAT

>KNP17MP653 *Ae. vexans* gr

TGGTTAGGAAGTCGATATCTAGAGTATGAGGCTTTGGGTTTTCTCAACC
AAGACCATTGGGTTTCACGTGACCATCTGCCATGCGGAGTGGGAGGCGTTGGT
GTGAACTATTTTGGTAATTATCTGAAGGAAAT

>SHI17MP544 *Aedes* spp.

TGGCTGGGAAGTCGATATCTGGAATATGAGGCCTTGGGCTTCCTCAACG
AAGACCATTGGGTAGCCCGTGAAAATTTCCCGGGTGGCGTGGGAGGTCTCGGC
GTAAACTACTTTGGCTATTACTTGTGAGAAAT

>KNP17MP639 *Aedes* spp.

TGGTTGGGCAGTAGGTTCTTGAGTATGAGGCCCTGGGGTTTTTAAATGAGGAT
CACTGGGTAGCACGGGAGAATTTCCCTGGTGGTGTCCGAGGCATGGGCGTTAA
CTACTTTGGCTATTACCTGGAGGAAAT