

Universal Primers as a Potential Tool for the Detection of Emerging Flaviviruses

Sir,
Flaviviruses (FVs) are arthropod-borne viruses that affect humans in tropical regions worldwide.^[1] Previous reports^[1,2] have shown the importance of implementing FV novel detection strategies in tropical regions, such as the use of universal primers that enable a simultaneous identification of these pathogens in a single polymerase chain reaction (PCR).^[1-3]

We analyzed 462 blood samples from febrile patients in western Mexico. FV identification was carried out through a PCR assay using universal primers validated *in vitro* by Maher-Sturgess *et al.* A total of 74 blood samples amplified with different amplicon sizes similar to those previously described.^[4] Our DNA sequencing analysis showed the identification of dengue virus serotype 1 in 31 blood samples,^[5] and the remaining 43 amplicons corresponded to different human gene regions [Table 1], representing 9.3% of all the samples analyzed.

Undoubtedly, the use of nonvalidated universal PCR primers in natural hosts represents a risk for obtaining unreliable results in animal and human populations due to the diversity of genomes. The development of universal PCR primers for FV detection is a potential tool for its diagnosis, especially in tropical regions where FVs frequently coexist, so febrile symptoms associated with FVs easily could result in a diagnostic confusion. This clinical confusion could interfere on the deployment of effective solutions for the detection, prevention, and control of emergent FV outbreaks in timely manner; therefore, the control of FV diseases could be hampered if effective and reliable detection strategies are not implemented.

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Table 1: Polymerase chain reaction results using the universal primers, Flav100F and Flav200R, corresponding to different sizes of amplicons of human genes

Number of samples	Description	Query position	Amplicon length (bp)	Query cover (%)	E	Accession number
8	<i>Homo sapiens</i> mitochondrial ND1 gene*	191-867	~690	~98	0.0	LC178870.1
1	<i>Homo sapiens</i> isolate XEB057 mitochondrion	3496-4236	758	97	0.0	KF056243.1
1	<i>Homo sapiens</i> chromosome 15 clone RP11-353B9 map 15q21	67,269-67,987	735	97	0.0	AC018927.6
1	<i>Homo sapiens</i> ELP4	175,278-174,496	796	98	0.0	NG_034086.1
1	<i>Homo sapiens</i> isolate GranChaco16 haplogroup B2 mitochondrion	3496-4172	691	97	0.0	KP172434.1
1	<i>Homo sapiens</i> EHBP1	195,453-196,134	704	96	0.0	NG_016758.1
1	<i>Homo sapiens</i> ROBO1	282,692-283,334	651	98	0.0	NG_011729.1
1	<i>Homo sapiens</i> DPYD	768,218-768,819	622	96	0.0	NG_008807.2
1	<i>Homo sapiens</i> chromosome 8, clone RP11-449M6	109,136-109,669	553	96	0.0	AC090574.6
1	<i>Homo sapiens</i> FAM189A2	39,721-403,20	615	97	0.0	NG_052888.1
1	<i>Homo sapiens</i> isolate HGDP00234 mitochondrion	3496-4171	693	97	0.0	KP240920.1
1	<i>Homo sapiens</i> SGCD	626,147-626,990	851	99	0.0	NG_008693.2
1	Human DNA sequence from clone RP11-138I18 on chromosome 10	20,188-21,041	871	98	0.0	AL512631.11
1	<i>Homo sapiens</i> isolate mtGHispPR0007 mitochondrion	3492-4168	691	97	0.0	KM102107.1

Contd...

Table 1: Contd...

Number of samples	Description	Query position	Amplicon length (bp)	Query cover (%)	E	Accession number
1	<i>Homo sapiens</i> chromosome 1 clone RP11-84O12	137,829-138,704	893	98	0.0	AC099670.2
1	Human DNA sequence from clone GS1-115G20 on chromosome 1	148,903-149,647	764	97	0.0	AL078645.31
1	Human DNA sequence from clone RP11-541G7 on chromosome X	31,561-32,248	706	97	0.0	AL590410.13
1	<i>Homo sapiens</i> apolipoprotein L, 3, mRNA	798-1432	649	97	0.0	BC042918.1
1	<i>Homo sapiens</i> ERBB4	532,004-532,840	844	99	0.0	NG_011805.1
1	<i>Homo sapiens</i> genomic DNA, chromosome 11q, clone: CMB9-14B22	60,374-60,956	594	98	0.0	AP000484.5
1	<i>Homo sapiens</i> steroid-5-alpha-reductase, alpha polypeptide 1 (3-oxo-5 alpha-steroid delta 4-dehydrogenase alpha 1) (SRD5A1) gene	23,462-24,192	751	97	0.0	AY341029.1
1	<i>Homo sapiens</i> BAC clone RP11-421J10	15,356-15,933	602	96	0.0	AC009236.4
1	<i>Homo sapiens</i> isolate HGDP00234 mitochondrion	3495-4171	688	98	0.0	KP240920.1
1	<i>Homo sapiens</i> FAM189A2, RefSeqGene on chromosome 9	39,721-40,318	615	97	0.0	NG_052888.1
1	Human DNA sequence from clone RP11-420G9 on chromosome 1	1532-1905	385	97	0.0	AL358013.19
1	Human DNA sequence from clone RP13-766B11 on chromosome X	18,172-18,595	434	97	0.0	BX510661.4
1	<i>Homo sapiens</i> BAC clone RP11-717F1 from 16	1021-1567	558	98	0.0	AC079801.2
1	Human DNA sequence from clone RP11-327L9 on chromosome 9	34,895-35,445	567	97	0.0	AL161782.14
1	<i>Homo sapiens</i> BAC clone RP11-354N12 from 2	80,765-81,132	381	96	0.0	AC009960.10
1	<i>Homo sapiens</i> LAMA2	12,902-13,384	502	96	0.0	NG_008678.1
1	Human DNA sequence from clone RP11-267I18 on chromosome 13	60,062-60,535	490	96	0.0	AL162852.14
1	Human DNA sequence from clone RP11-49A19 on chromosome 13	20,879-21,452	582	98	0.0	AL354741.16
1	<i>Homo sapiens</i> chromosome 1 clone RP11-335E6	71,818-72,229	431	95	0.0	AC096543.2
1	<i>Homo sapiens</i> isolate mtGHispTX0026 mitochondrion	3496-3870	386	97	0.0	KM102130.1
1	<i>Homo sapiens</i> 3 BAC RP11-561L3 (Roswell Park Cancer Institute Human BAC Library)	16,361-16,723	376	96	0.0	AC092969.7
1	<i>Homo sapiens</i> UTRN, RefSeqGene on chromosome 6	22,2195-222,599	424	95	0.0	NG_042293.1

*Sequence analysis of these amplified segments showed a similarity variation of ~4 bp. ELP4: Elongator acetyltransferase complex subunit 4, EHP1: EH domain-binding protein 1, ROBO1: Roundabout guidance receptor 1, DPYD: Dihydropyrimidine dehydrogenase, FAM189A2: Family with sequence similarity 189 member A2, SGCD: Sarcoglycan delta, ERBB4: Erb-B2 receptor tyrosine kinase 4, LAMA2: Laminin subunit alpha 2, UTRN: Utrophin

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Conflicts of interest

There are no conflicts of interest.

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