Do-It-Yourself PCR: Serotyping Dengue Viruses in *Aedes* Mosquitoes Within El Salvador

India Grace, Cory Dickson and Anna M. Groat Carmona*

Division of Sciences and Mathematics | University of Washington Tacoma | Tacoma, WA

Dengue Surveillance in *Aedes* Mosquitoes

Welcome to the Family *Flaviviridae!*

Flaviviridae is a viral family that includes notorious public health pathogens such as Zika virus (ZIKV), Yellow Fever virus (YFV), and Dengue virus (DENV). DENV is the causative agent of Dengue fever, also known as breakbone fever, and is transmitted by *Aedes aegypti* mosquitoes (Figure 1). DENV consists of four serotypes (DENV1-4) and is particularly hard to manage because getting infected a second time by a heterologous serotype more often leads to severe Dengue, complicating vaccination efforts.

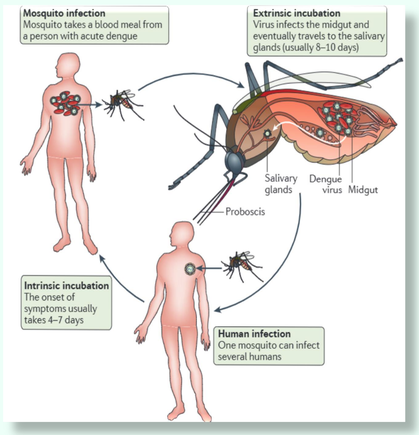


Figure 1. The life cycle of DENV1-4 within its human host and *Aedes* mosquito vector (Guzman 2016).

Mosquito Focused vs. Patient Focused

A good, robust healthcare system includes surveillance of vector-borne pathogens in both their human hosts and their arthropod vectors. While Dengue cases are reported to the Ministry of Health from public hospitals, most infections are clinically asymptomatic. Additionally, even in cases where symptoms are present, patients may not go to the hospital for aid (Figure 2). Solely focusing on patient incidences will only reflect a small view of the disease incidence and its effects on the population. To have a full picture, we must also monitor *Aedes* mosquitos to know where DENV is present and what serotypes of DENV (1-4) are currently circulating within El Salvador.

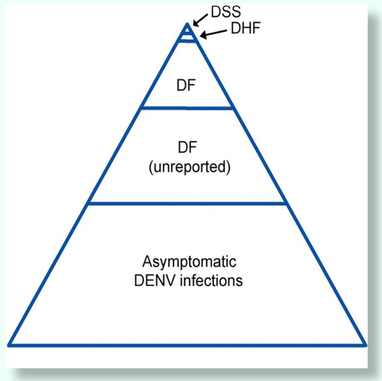


Figure 2. Schematic depiction of the pyramid-like nature of DENV clinical manifestations. Obtained from personal communications with Dr. Eva Harris.

Why Should You Care?

Annually, 100 million people get infected with DENV1-4 (*Dengue and Severe Dengue* 2024) and due to global climate change, that number will likely increase as *Aedes*' territory stretches into the northern hemisphere (Figure 3).

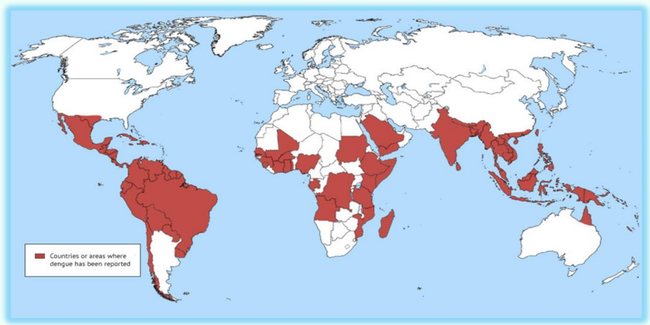


Figure 3. Areas where Dengue infections have been reported globally (red) (*Interesting Facts about Aedes* 1970).

DIY Experimental Road Map

Rebuilding Together

The civil war (1972-1992) in El Salvador destabilized the region (*Salvadoran Civil War* 2024). Currently, local health care systems are rebuilding, and the overarching goal of this project is to help support the continued success of their efforts to surveil vector-borne pathogens. Working with community partners, including the Universidad Centroamericana José Simeón Cañas (UCA) and the Universidad de El Salvador (UES), we hope to establish a Dengue surveillance program in field-caught *Aedes* mosquitoes collected throughout the region (Figure 4). In conjunction with the Ministry of Health in El Salvador, we hope to learn more about the incidence of Dengue within the country.

Figure 4. Map of El Salvador and its 14 municipalities. Stars indicate areas where *Aedes* mosquitoes are actively being collected by scientists at the Universidad de El Salvador in collaboration with the Ministry of Health (*Provinces Map of El Salvador*).

"Kit-Free" PCR Protocol

Due to fiscal restrictions, traditional pre-made molecular kits cannot easily be shipped into El Salvador, so we sought to make a "kit-free" semi-nested PCR-based protocol that would enable researchers to test for the presence of DENV and identify what serotypes of Dengue (DENV1-4) are circulating in the field-caught *Aedes* mosquitoes at that time (Figures 5 and 6). Adding this layer of disease surveillance to existing programs will help lower the rate of Dengue incidence in El Salvador, empowering local government efforts to actively monitor the disease more effectively. This protocol can be similarly implemented in other Latin American countries where Dengue is becoming a growing concern due to climate change (Figure 3). Luckily, the local Ministry of Health is already collecting *Aedes* mosquitoes (Figure 4) though they currently do not test for vector-borne pathogens, which is where our protocol comes into play (Figure 6).

Acknowledgments and References

Thank You!

We would like to acknowledge the Puyallup and Muckleshoot Tribes for protecting the land where we work and go to school. We would also like to thank Dr. Anna M. Groat Carmona as well as our other collaborators, including the Universidad de El Salvador, the Universidad Centroamericana and the Ministry of Health in El Salvador.

Designing DENV Primers

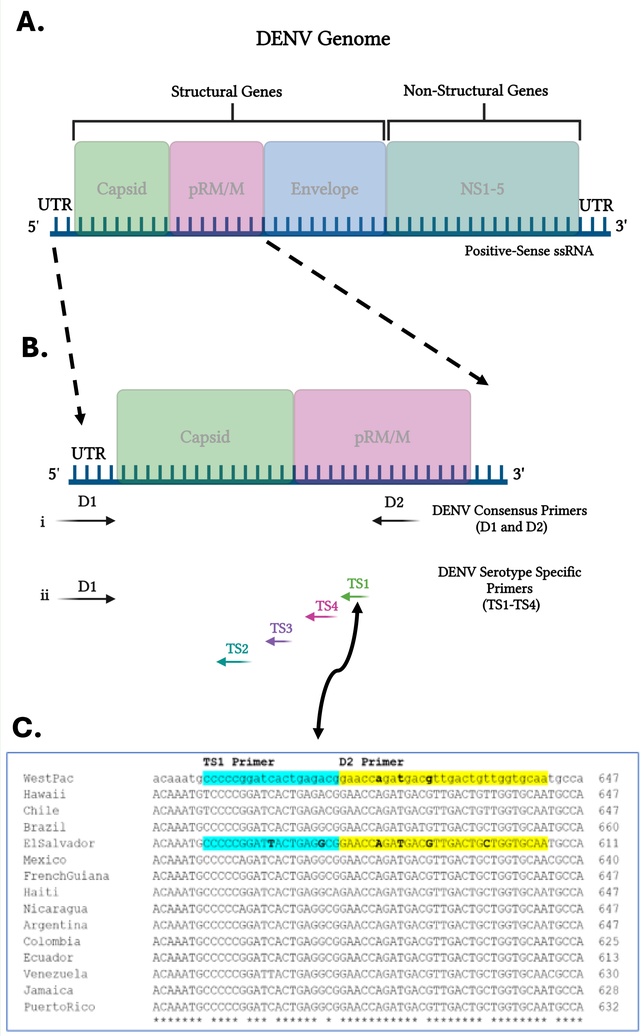
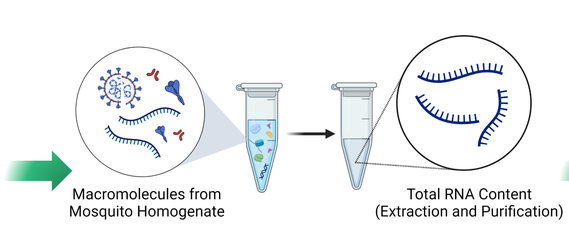


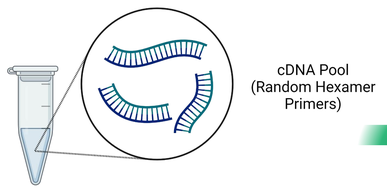
Figure 5. Designing DENV primers for the Semi-Nested PCR Protocol. A) The DENV genome (~10.7 Kbp) and its corresponding genes. B) The DENV consensus primers (D1 and D2), which indicate if DENV genome is present within mosquito sample as well as DENV serotype specific primers (TS1-TS4), which indicate what serotype (DENV1-4) is present. C) An example of DENV 1 sequence alignment, DENV consensus primer (D2) and DENV serotype specific primer (TS1) were designed by comparing conserved sections of the DENV genome from strains isolated from multiple Latin American countries (Made with Biorender).

Semi-Nested PCR Protocol

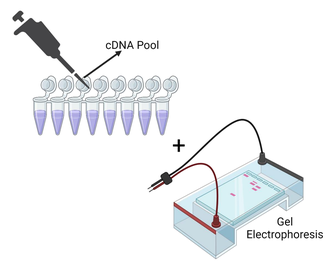
A. Mosquito homogenate from field-caught *Aedes aeygpti:*
Making a "skeeter shake" before total RNA extraction and cDNA synthesis can be preformed.



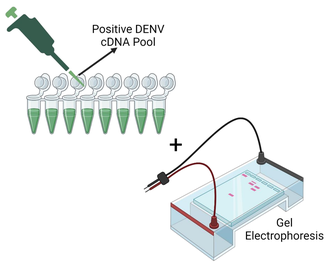
B. RNA Extraction:
Total RNA content is extracted from the mosquito homogenate and purified to act as a temple for the cDNA synthesis.



C. cDNA Synthesis:
Reverse transcription of the purified RNA extract to create a complementary DNA (cDNA) for the semi-nested PCR assay.



D. PCR #1 + Gel Electrophoresis:
Testing the mosquito homogenate cDNA pool to see if DENV genome is present within the field-caught *Aedes* mosquito samples. If DENV genome is present (positive DENV cDNA pool), then the second PCR can be preformed.



E. PCR #2 + Gel Electrophoresis:
DENV positive cDNA samples are subjected to a second PCR to see what DENV serotypes are present (DENV1-4).

Figure 6. "Kit-free" Protocol (steps A through E) to identify DENV1-4 from field-caught *Aedes* mosquitoes (Made with Biorender and *Premium Photo*).