Characterization of conserved regulatory RNA elements in the coding-region of the Dengue viral genome

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Dengue virus (DENV) is one of the most significant mosquito-borne viruses that affects humans and is transmitted by *Aedes aegypti* and *A. albopictus* mosquitoes. DENV is an enveloped positive-sense, single stranded RNA virus that belongs to the *Flaviviridae* family, and its genome encodes for 10 viral proteins in a single open reading frame (ORF) flanked by highly structured 5' and 3' untranslated regions (UTRs). The conserved RNA structures and sequences within the UTRs are known to play key roles in regulating the viral life cycle, including directing the synthesis of its RNA genome and regulating translation of the viral ORF. However, little is known about the conserved RNA elements within the coding-region. Previously, four putative RNA elements were identified using various bioinformatics platforms: two sequence elements in nonstructural (NS) coding-regions (NS3 and NS5), and two structural elements in capsid. Overlap extension PCR was performed to introduce silent mutations into these regions of the viral genome, and the resulting amplicons were subcloned into an intermediate vector (pGEM-T). We are currently transferring the mutant amplicons into an infectious viral cloning vector (pD2/IC), which we can use to generate mutant viruses and better understand the role that coding-region RNA elements have within the DENV life cycle. It is important to identify regulatory RNA elements that might exist within the coding-region of the DENV genome to further characterize regulation of the viral life cycle. Enhancing our understanding of the mechanisms that influence DENV replication provides insight for the development of novel antiviral drug targets.

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