Mutagenesis of Putative RNA Sequence Elements within the Dengue Virus Genome

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Abstract

Dengue fever is caused by a mosquito-borne flavivirus, resulting in a debilitating and potentially fatal illness affecting millions globally. The dengue virus (DENV) serogroup includes four distinct serotypes (DENV1–4), but currently there are no specific antiviral treatments for the disease. Understanding flavivirus replication is key to developing effective therapies. Previous bioinformatic studies identified putative RNA sequence elements that were conserved across DENV1-4, suggesting they play a vital role in viral replication. To test this, silent mutations were introduced to disrupt two conserved sequences within the DENV2 genome: Conserved Protease Coding-Region 1 (CPCR-1) in the NS3-coding region and Methyltransferase RNA-Dependent Coding-Region 1 (MRdCR-1) in the NS5-coding region. Mutant DNA amplicons were created using overlap-extension PCR and subcloned into a pGEM-T cloning vector for propagation in *Escherichia coli*. However, integration of the proposed mutations remains inconclusive based on the diagnostic digests and sequencing results obtained. Once confirmed, these mutant amplicons will be transferred into a DENV2 infectious cloning vector (pD2/IC) to generate mutant viral RNAs (vRNA). The resulting vRNAs can then be transfected into mammalian host cells and tested using functional assays to assess the impact of these mutations on viral replication. Ultimately, this research aims to identify conserved RNA elements that are important for viral survival, paving the way for novel antiviral treatments for dengue fever.