Analysis of putative RNA structural element from the dengue virus type 2 capsid-coding region

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Dengue virus (DENV) is a positive-strand RNA virus that is transmitted by *Aedes* mosquitoes and is part of the family Flaviviridae, which includes Japanese encephalitis virus (JEV) and tick-borne encephalitis virus (TBEV). Silent mutations were introduced into a region of interest (ROI) called the viral capsid-coding sequence (VCCS), which was found to be a highly conserved structure throughout the DENV1-4, JEV, and TBEV serogroups using sequence alignments and structure predictions. Conserved RNA structures have the potential to be regulatory. Mutant amplicons were previously generated using overlap extension-PCR, which introduced silent mutations into the ROI (VCCSmut1 and VCCSmut2). Mutant ROIs were ligated into an intermediate vector (pGEM-T). Mutant pGEM-T constructs were introduced to *Escherichia coli* for propagation, then extracted and evaluated for ROI presence using diagnostic restriction digest (SalI) and sequencing. VCCSmut1 and a DENV2 infectious cloning vector (pD2/IC) were subjected to a subcloning restriction digest (SacI and SphI). Purified inserts (VCCSmut1) were ligated into the digested pD2/IC vector and propagated in *E. coli* for extraction and evaluation. Next steps are to subclone the VCCSmut2 mutant insert from pGEM-T into pD2/IC, as our goal is to compare the phenotypes for both VCCS mutants when corresponding viral RNAs are transfected into mammalian cells and assessed using a plaque assay. The potential of VCCS acting as an RNA structural element that regulates viral replication strengthens our understanding of DENV replication and contributes to broader efforts to develop antiviral treatments for dengue fever by revealing new drug targets.