Generation of Mutant DENV2 Infectious Clones

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Abstract:

Dengue virus (DENV) is a mosquito-borne flavivirus that poses a significant public health threat in tropical and subtropical regions worldwide. There are four distinct serotypes (DENV1-4), each capable of causing dengue fever and, in some cases, severe dengue. Flavivirus replication requires cyclization of its single-stranded RNA genome, a process regulated by three conserved RNA sequence elements located in the 5' and 3' untranslated regions. The viral capsid-coding structure (VCCS), located within the capsid-coding region, was previously identified as a conserved RNA structural element in DENV1-4. Silent mutations were introduced into the DENV2 (Thailand, strain 16681) infectious clone plasmid (pD2/IC) utilizing overlap extension PCR, to disrupt either the predicted structure or primary sequence of VCCS, and determine whether it plays a functional role in viral replication. Mutagenized amplicons were cloned into an intermediate pGEM-T vector, before being transformed into competent *Escherichia coli* (DH5α strain). A diagnostic digest was subsequently performed on the extracted pGEM-T constructs, followed by gel electrophoresis to confirm the presence of our mutagenized amplicons. The results from the diagnostic digest indicated that we were unable to incorporate our mutagenized VCCS amplicons into the pGEM-T vector. Assuming we are able to complete the pGEM-T cloning procedure, our next steps would require subcloning our mutagenized fragments into the final pD2/IC vector to generate viral RNAs that can be tested using plaque assays to see if VCCS is important for viral replication. This research furthers our understanding for how RNA elements regulate DENV replication, which could inform future therapeutic strategies.