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

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Abstract

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.

Virology

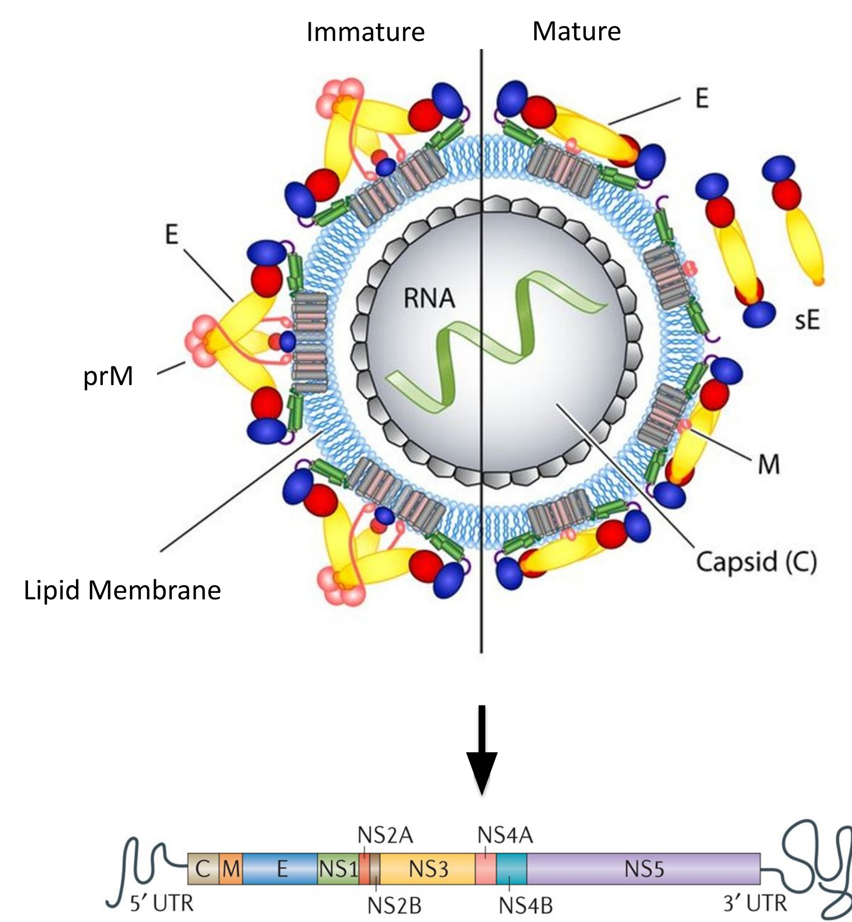

Figure 1. Structure of dengue virion and ssRNA genome. DENV is an enveloped virus, roughly 50 nanometers in diameter. The viral genome is a positive-sense ssRNA that encodes for 10 viral proteins in a single open reading frame (ORF). The virus encodes for structural proteins (capsid-C, membrane-prM/M and envelope-E). The other seven genes are nonstructural (NS) proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) involved in viral replication. Images taken from Guzman (2016).

Homology Table

Tables 1 and 2. Homology calculations for proposed RNA sequence elements located within the NS3 (CPCR-1) and NS5 (MRdCR-1) coding-regions from the DENV 1-4, Japanese encephalitis virus (JEV) and tick-borne encephalitis (TBEV) serogroups.

Conserved Protease Coding Region 1 (NS3)

Serogroup	# Of Similar Base Pairs	% Similarity
DENV 1-4	13/14	93%
JEV	8/14	57%
TBEV	9/14	64%

Metyltransferase RNA-dependent Coding Region 1 (NS5)

Serogroup	# Of Similar Base Pairs	% Similarity
DENV 1-4	23/27	85%
JEV	--	--
TBEV	19/27	70%

Conserved RNA Sequence Elements

Figure 2. Depiction of previously designed mutations in putative conserved RNA sequence elements. Silent mutations were designed for the RNA sequence elements in NS3 and NS5 coding-regions (CPCR-1 and MRdCR-1 respectively). Mutations were designed so as to not alter the wild type amino acid sequence but disrupt primary the nucleotide sequence.

DENV 1-4 Consensus Structure (5′ UTR + Capsid)

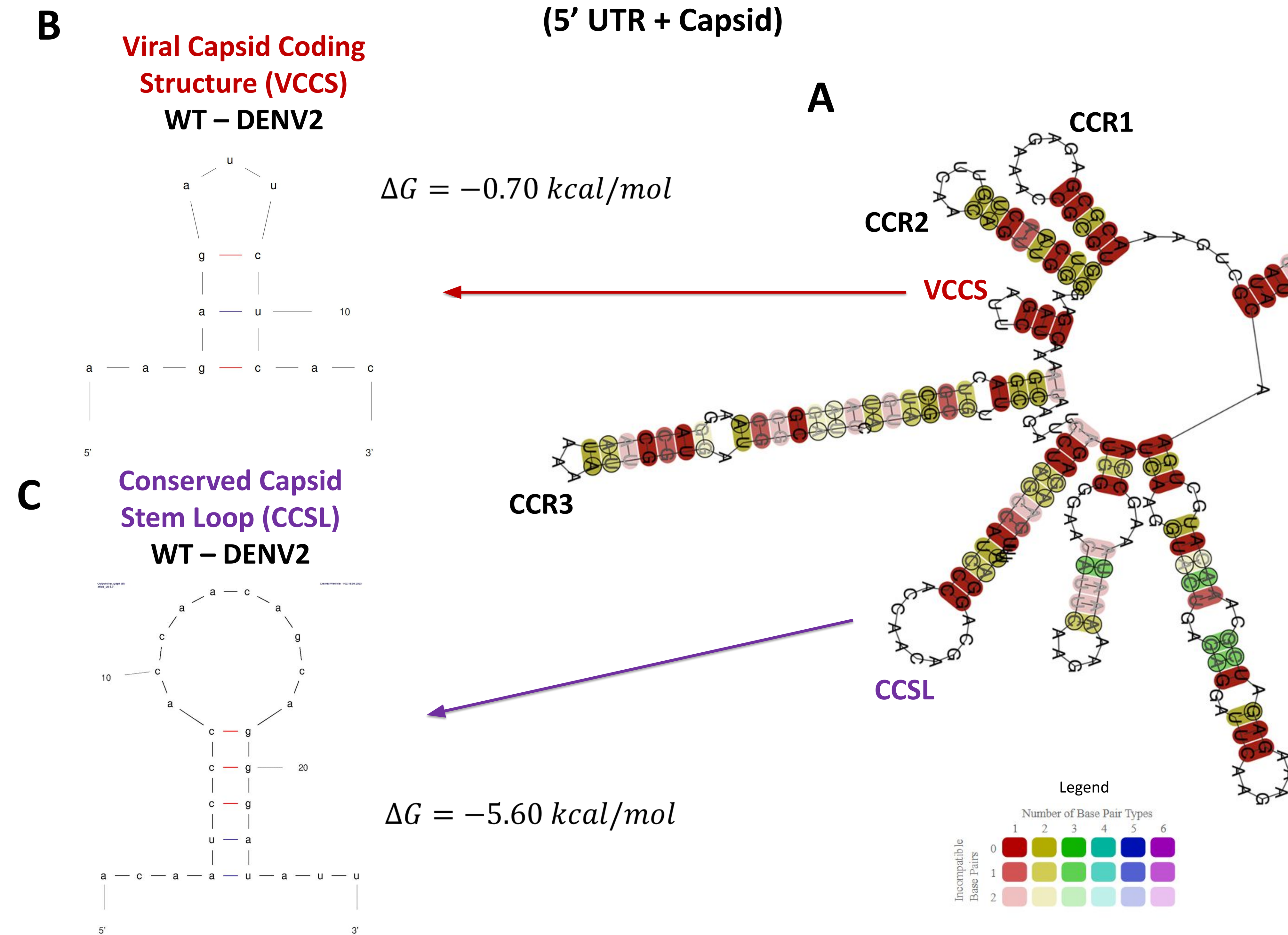

Figure 3. Depiction of wild type RNA structural elements (viral capsid coding region [VCCS] and conserved capsid stem loop [CCSL]) within the capsid coding-region of DENV. Image of conserved RNA structures were generated using RNAalifold and sequence alignments for the DENV 1-4 (A), Japanese encephalitis virus and tick borne encephalitis virus serogroups (data not shown). The putative wildtype RNA structures in DENV2 for VCCS (B) and CCSL (C) were generated using RNAfold.

Mutating RNA Structure Elements

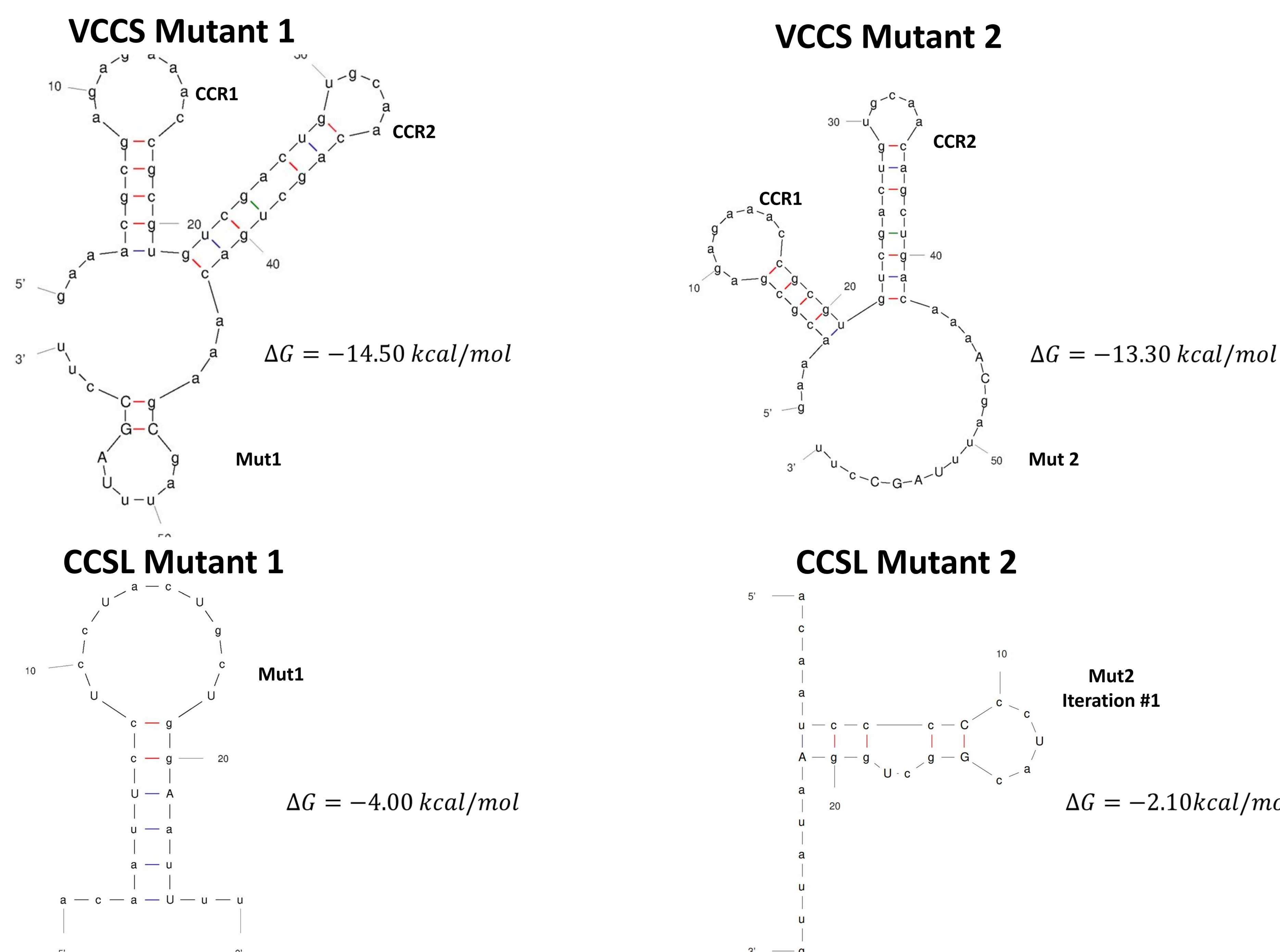

Figure 4. Depiction of mutated RNA structural elements within the capsid coding-region of DENV2. Images of mutated RNA structure elements were generated using mFold. For each putative RNA structure, 2 mutants were designed using silent amino acid changes. One mutant (mut 1) maintained putative RNA structure while disrupting the primary nucleotide sequence while the other (mut 2) had disruptions to both the proposed secondary structure and primary nucleotide sequence.

Methods

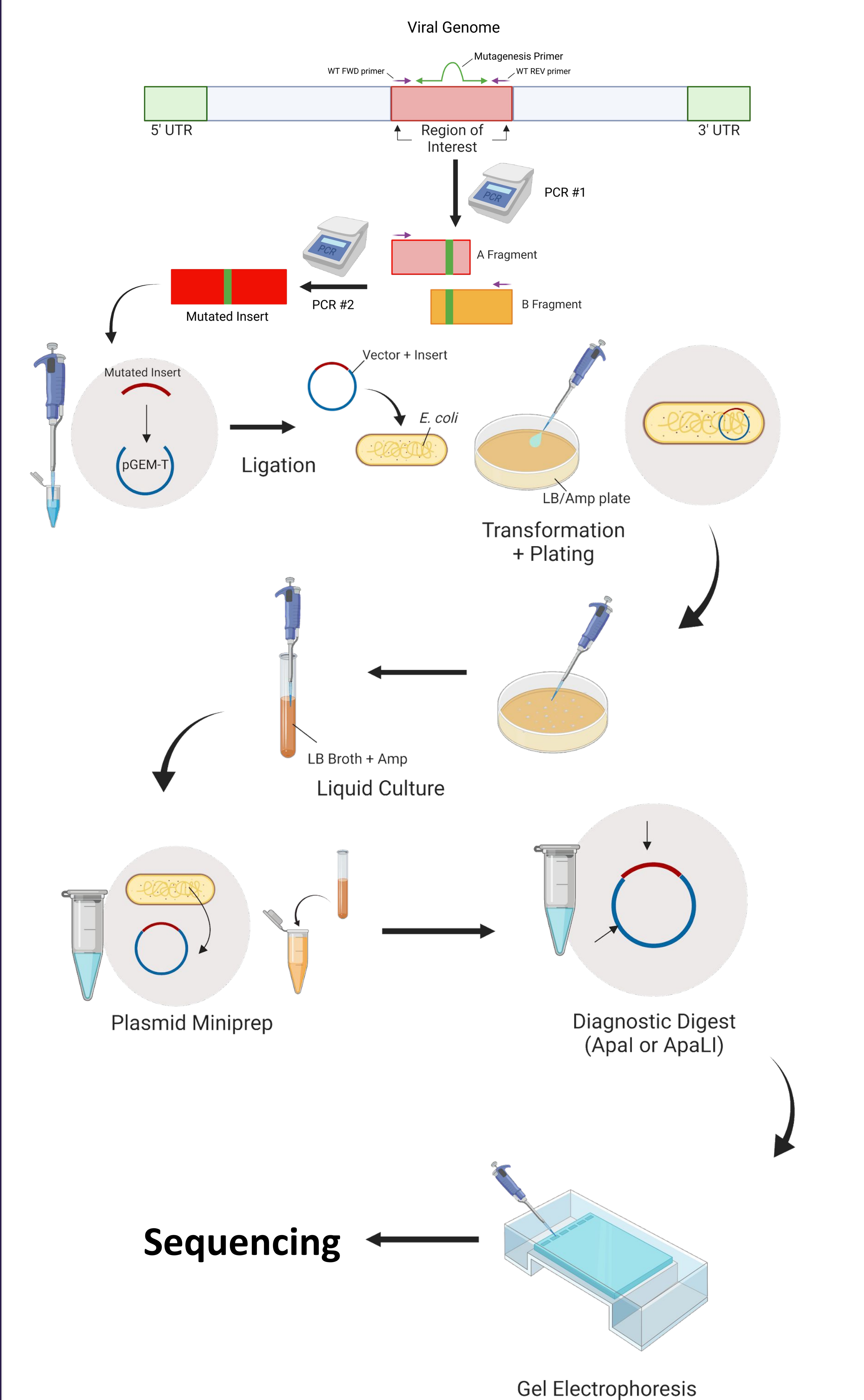

Figure 6. Graphical representation of methods utilized to generate mutated regions of interest. Overlap extension PCR was used to generate mutated inserts that were ligated into an intermediate vector (pGEM-T). The vector was then transformed and plated in *E. coli* on LB plates with 100 ug/mL ampicillin. Colonies were then grown in a liquid culture containing LB broth and 100 ug/mL ampicillin followed by a plasmid miniprep to isolate the plasmid. Diagnostic restriction digest was performed to screen the plasmid, followed by gel-electrophoresis and sequencing for verification of the mutated inserts.

Future Directions

Our next step is to verify the mutations we made were successfully introduced using diagnostic restriction digest and gel electrophoresis. Successful results will be confirmed by sending the isolated plasmid DNA off for sequencing. Once verified, we can then subclone our mutated sequences into an infectious viral cloning vector where we can produce virus *in vitro* and observe the effect that these mutations have on the life cycle of DENV. These results can provide us with information as to the potential function of each of the conserved coding-region RNA elements. Therefore, understanding the function of these conserved coding-region RNA elements allows for greater insight towards the development of novel antiviral drugs.

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