Exploring the role of *Aedes aegypti* cysteine rich secretory protein venom allergen V3 in dengue virus infection.

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**INTRODUCTION**

Dengue virus (DENV), a flavivirus causing dengue fever, infects 400 million people annually (1). Vectored by the Aedes aegypti mosquito, there is currently no vaccine for DENV (1). One strategy to find possible vaccine targets for flaviviruses is to analyze the functions of specific molecules involved in the infection and transmission processes (2). Studies have demonstrated a potent role of *Aedes aegypti* exosomes in the transmission of DENV (3). We looked into the protein contents of Aedes aegypti exosomes and selected Cysteine rich venom protein 793 (CRVP793) V3 Allergen as it is very similar to cysteine rich protein venom allergen (AAEL000379) that was found to be necessary for DENV infection in mosquitoes (4). CRVP793 V3 Allergen protein was also found in mosquito’s saliva, thus making it an interesting target to further explore.

**METHODS & MATERIALS**

**Flow diagram**

- Amplification
- Cloning confirmation
- Confirmation of gene insert in both plasts-plb-MCS and pmT-V5-His vectors by Sanger sequencing.
- Restriction enzymes mediated cloning method to insert the gene into the plasts-plb-MCS and pmT-V5-His vectors.
- Polymerase chain reaction to amplify the AAEL000793 gene from Aedes aegypti.

**Mosquitoes used in study**
- Aedes aegypti-Rockefeller strain.

**RNA extraction and cDNA synthesis**
- RNA extracted from mosquitoes using RNaseasy miniKit (Qiagen).
- 2ug of RNA used to synthesize complementary DNA (cDNA) using oligo-dT primer.

**Gene Cloning**
- Amplified CRVP-793 allergen V3 gene using gene specific primer.
- Cloned into pmT-V5-His and plasts plb-MCS vector.

**Cloning confirmation**
- Restriction digestion
- Sanger sequencing

**DATA & RESULTS**

**1. Sequence Analysis**

![Figure 1a: Restriction mapping of CRVP793 V3 allergen using NEBcutter.](image)

Figure 1a: Restriction mapping of CRVP793 V3 allergen using NEBcutter.

**2. Amplification CRVP793 V3 Allergen**

![Figure 2: Gel image of amplification of CRVP793 V3 Allergen from mosquitoes and cell line Aag2.](image)

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**3. Cloning of CRVP793 V3 Allergen into pmT/V5-His and plsfal-pUb-MCS**

![Figure 3a: Resultant colonies after transformation of ODH5a bacteria.](image)

Figure 3a: Resultant colonies after transformation of ODH5a bacteria.

**4. Confirmation of CRVP793 V3 Allergen Cloning**

![Figure 4a: Positive clones were confirmed from a gel image of a restriction digestion of CRVP793 V3 allergen with the restriction enzymes BglII and XbaI.](image)

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**5. Ammonium chloride precipitation**

- Precipitation of allergen
- Purification of allergen

**REFERENCES**

Citations: