Abstract No: STMM 07 Science, Technology, Mathematics and Medicine

CRISPR/cas9 mediated gene knockout in mosquito vector *Aedes aegypti*: *In-silico* approach

P. V. D. Dilani[1,3] D. L. P. E. Gunasekara[2], A. B. Carrillo- Gálvez [3], M. Hapugoda[4], C. M. Hettiarachchi[1], R. S. Dassanayake[1], F. Zurita[3] and Y. I. N. S. Gunawardene[4*]

[1]Department of Chemistry, Faculty of Science, University of Colombo, Sri Lanka
[2]Sri Lanka Institute of Biotechnology (SLIBTEC), Homagama, Sri Lanka
[3]Department of Genetics, University of Granada, Granada, Spain
[4]Molecular Medicine Unit, Faculty of Medicine, University of Kelaniya, Ragama, Sri Lanka

Aedes aegypti is the principal vector of several viruses including Dengue, Chikungunya and yellow fever. The recent advent of Clustered-Regularly-Interspaced-Short-Palindromic-Repeats (CRISPR)-Cas9 has sparked significant enthusiasm for the genetic control of *A. aegypti*. In the current study, we hypothesized that CRISPR-cas9 mediated gene knockout of doublesex *(Aedsx*) and Actin-4 (*AeAct-4)* in *A. aegypti* would result in sterile intersex phenotype and flightless phenotype respectively in females. Designing effective sgRNA is the most critical part of CRISPR/Cas9 editing. Therefore, sgRNAs were designed *in-silico* with the aim of developing efficient measures to control *A. aegypti* populations. Gene sequences of *Aedsx* (IAAEL009114) and *AeAct-4* (AAEL001951) were retrieved from sequence data available in the VectorBase database. BLASTP was performed in searching for paralogs. Phylogenetic analysis was performed for all paralogs with ≥80% amino acid similarity. Eight paralogs were identified for *the AeAct-4* while none was identified for *Aedsx.* DNA sequences for the paralogs of *AeAct-4* were aligned to identify targets for sgRNAs that were unique to *AeAct-4.* Exon 2 was selected for *AeAct-4*, as the target site. For the *Aedsx* gene, 5a and 5b female-specific exons were selected as the target sequence for *AedsxF1* and *AedsxF2* isoforms respectively. sgRNAs were designed using online tools CHOP CHOP and CRISPR guide express. Two sgRNAs for each *AeAct-4* gene, *AedsxF1* and *AedsxF2* isoforms with minimum off-target effects and *in-silico* predicted efficiency > 60% were selected to maximise the on-target gene knockout *in-vivo.* The efficiency of gene knockout can be evaluated by microinjecting synthesized sgRNAs directly into mosquito embryos with Cas9protein.

Keywords: *Aedes aegypti*, CRISPR/Cas9, Population suppression, sgRNA

*nilminisg@kln.ac.lk