Table 1 Mutagenic primers for site-directed mutagenesis

Primer	Sequence (5'→ 3')
I139AF	5' GAAGCTACAGCTAAAGGCACATTTAC 3'
S159AF	5' GGATTTTTTGGCATGCTTTATCCGCCC 3'
L160AF	5' GGCATAGTGCATCCGCCCACAATACAA 3'
S161AF	5' GGCATAGTTTAGCCGCCCACAATACAAG 3'
A162VF	5' GGCATAGTTTATCCGTACACAATACAAG 3'
D209NF	5' CAATAAAAAATAGTGCACGATATGAAG 3'
V215AF	5' GTGCACGATATGAAGCTAAAATGAAAGC 3'

Primers were designed based on cyt2Aa2gene (GenBank Accession No. AF472606). Mutated nucleotides were underlined. This table showed only the forward primers. DNA sequences of the reverse primers are complementary to their forward primers

Table 2 Mosquito-larvicidal and hemolytic activities of Cyt2Aa2 wild type and mutants against *A. aegypti* larvae and RBC.

Toxin	Protein Expression	Solubility	Activation	Hemolytic end-point (µg/ml)	Larvicidal activity LC_{50} (µg/ml)
WT	Yes	+++	Yes	0.05-0.1	0.6 (0.5-0.7)
I139A	Low	_	nd	nd	Non toxic
S159A	Yes	+++	Yes	0.1-0.2	1.3(1.0-1.6)
L160A	Yes	+++	Yes	0.1-0.2	1.3 (1.0-1.6)
S161A	Yes	+++	Yes	0.2-0.4	1.7 (1.2-2.2)
A162V	Yes	+++	Yes	No lysis	Non toxic
D209N	Yes	+++	Yes	No lysis	1.3 (0.9-1.7)
V215A	Yes	_	nd	nd	Non toxic

Solubility was determined by incubating the toxin inclusions in 50 mM Na_2CO_3/$NaHCO_3$, pH 10.5, plus 10 mM at 37C for 1 h. The soluble proteins were activated by proteinase K (1%, w/w) at 37^{o}C for 1 h. Hemolysis was tested against rat red blood cells and mosquito larvicidal activity was assayed using *A. a*egypti larvae. The assays were repeated three times. *nd, not determined because there was no activated toxin for the assay.