TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENT	iii
ABSTRACT (IN ENGLISH)	v
ABSTRACT (IN THAI)	viii
TABLE OF CONTENTS	x
LIST OF TABLES	xiv
LIST OF FIGURES	xvi
ABBREVIATIONS	xxi
CHAPTER I INTRODUCTION	1
1. Problems and research rationale	1
2. Literature review	5
2.1 Insecticide resistance mechanisms	5
2.2 Mammalian voltage-gated sodium channels	6
2.3 Insect voltage-gated sodium channels	14
2.4 Insect sodium channel gene and knockdown resistance	17
2.5 The simplified high throughput methods	24
3. Purposes of this study	e ₂₇
4. Significant of the research	28

Х

TABLE OF CONTENTS (continued)

			Page
CH	IAP	TER II MATERIALS AND METHODS	29
	1.	Mosquito strains	29
	2.	Mosquito rearing	29
	3.	Insecticide susceptibility test	30
		3.1 Insecticide susceptibility test for larvae	30
		3.2 Insecticide susceptibility test for adult mosquitoes	31
	4.	Genetic inheritance of the F1534 and C1534 alleles in permethrin	31
		susceptible and resistant Ae. aegypti	
	5.	Bioassay data analysis	32
	6.	Isolation of total RNA and genomic DNA from mosquito	34
		6.1 Total RNA extraction	34
		6.2 Genomic DNA extraction from single mosquito	34
	7.	Amplification of the Ae. aegypti voltage-gated sodium channel gene	35
		7.1 Amplification of the Ae. aegypti voltage-gated sodium	35
		channel gene using cDNA templates	
		7.2 Amplification of the Ae. aegypti voltage-gated sodium	39
		channel gene using Genomic DNA templates	
	8.	Direct sequencing and sequence analysis	42
	9.	Development of TaqMan SNP genotyping (TaqMan SNP) and	42
		AS-PCR assays for detection of the F1534C mutation in Ae.	
		aegypti	

TABLE OF CONTENTS (continued)

			Page
		9.1 TaqMan SNP Genotyping Assay	42
		9.2 Allele specific PCR	49
	10	. Genotyping of the F1534C mutation in wild caught Ae. aegypti	53
		by AS-PCR	
СНА	PT	ER III RESULTS	56
	1.	Partial cDNA sequencing of the Ae. aegypti voltage-gated sodium	56
		channel gene	
	2.	Sequence analyses of the Ae. aegypti voltage-gated sodium	64
		channel gene	
	3.	Genetic inheritance of the F1534 and C1534 alleles in Ae. aegypti	73
	4.	Development of TaqMan SNP genotyping assay	80
	5.	Development of Allele Specific PCR (AS-PCR)	84
	6.	Comparison of DNA sequencing with the TaqMan SNP and	86
		AS-PCR assays	
	7.	Distribution of the F1534C mutation in Ae. aegypti populations	88
	8.	Molecular variation of the IIP-IIS6 region of the Ae. aegypti	92
		voltage-gated sodium channel gene	

TABLE OF CONTENTS (continued)

	Pag	e
CHAPTER IV DISCUSSION	97	7
CHAPTER V CONCLUSION	104	4
REFERENCES	107	7
APPENDICES	124	4
Appendix A The RNA codons	125	5
Appendix B Amino acid abbreviations	126	5
Appendix C Chromatogram of nucleotide sequence	127	7
Appendix D Publications	140)

CURRICULUM VITAE

156

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่ Copyright[©] by Chiang Mai University All rights reserved

LIST OF TABLES

Table		Page
1.1	Neurotoxin that target the voltage-gated sodium	13
	channel and their corresponding receptor sites	
1.2	Sodium channel amino acid sequence	20
	polymorphisms associated with knockdown	
	resistance	
2.1	Sequences of primers for amplifying the Ae.	37
	aegypti voltage-gated sodium channel gene by	
	using cDNA as template	
2.2	Sequences of primers for amplifying the Ae.	41
	aegypti voltage-gated sodium channel gene by	
	using genomic DNA as template	
2.3	Sequences of TaqMan primers and probes for	48
	TaqMan SNP Genotyping Assay	
2.4	Sequences of oligonucleotides used for Allele	50
	Specific PCR Chiang Mail Unive	
2.5	Collection sites of Ae. aegypti in Thailand	e ⁵⁴ 0
3.1	Responses of the Ae. aegypti adults to 0.75%	75
	permethrin for the parental strains, their reciprocal	
	crosses and backcrosses	

LIST OF TABLES (continued)

Table		Page
3.2	Responses of the Ae. aegypti larvae to permethrin for	77
	the parental strains, their reciprocal crosses and	
	backcrosses	
3.3	Comparison of genotype results for the F1534C	87
	mutation obtained from the TaqMan SNP and AS-	
	PCR assays with DNA sequencing	
3.4	Frequency of the F1534C mutation in the Ae. aegypti	90
	voltage-gated sodium channel gene within dead and	
	survivor mosquitoes from 14 localities of Thailand	
	determined using the AS-PCR method	
3.5	Molecular variation of IIP-IIS6 region of sodium	94
	channel gene and the correlation with the F1534C	
	mutation in the Ae. ageypti permethin resistance from	
	Thailand	

Copyright[©] by Chiang Mai University All rights reserved

LIST OF FIGURES

Figure		Page
1.1	Schematic representation of the subunit structure of the	8
	voltage-gated sodium channel.	
1.2	The transmemebrane topology of the voltage-gated	9
	sodium channel	
1.3	Generation of an action potential	12
2.1	A schematic diagram of the extended transmembrane	38
	structure of voltage-gated sodium channel α subunits	
2.2	The principle of allelic discrimination using TaqMan	44
	SNP Genotyping Assay	
2.3	Schematic of the TaqMan SNP Genotyping for detection	47
	of the F1534C mutation	
2.4	Schematic of the Allele Specific PCR assays for	51
	detection of the F1534Cmutation	
3.1	Amplification of the Ae. aegypti voltage-gated sodium	57
	channel gene from PMD-R (A) and PMD (B) strains	
3.2	The cDNA sequences of the IS4 - IS6 domains of the	58
	Ae. aegypti voltage-gated sodium channel gene from	
	PMD-R and its deduced amino acid sequences	

xvi

xvii

LIST OF FIGURES (continued)

Figure		Page
3.3	The cDNA sequences of the IS4 - IS6 domains of the	59
	Ae. aegypti voltage-gated sodium channel gene from	
	PMD and its deduced amino acid sequences	
3.4	The cDNA sequences of the IIS1-IIS6 domains of the	60
	Ae. aegypti voltage-gated sodium channel gene from	
	PMD-R strains and its deduced amino acid sequences	
3.5	The cDNA sequences of the IIS1-IIS6 domains of the	61
	Ae. aegypti voltage-gated sodium channel gene from	
	PMD strains and its deduced amino acid sequences	
3.6	The cDNA sequences of the IIIS4-IVS2 domains of the	62
	Ae. aegypti voltage-gated sodium channel gene from	
	PMD-R strains and its deduced amino acid sequences	
3.7	The cDNA sequences of the IIIS4-IVS2 domains of the	63
	Ae. aegypti voltage-gated sodium channel gene from	
	PMD strains and its deduced amino acid sequences	
3.8	Alignment of the amino acid sequences of the Ae.	66
	aegypti voltage-gated sodium channel protein, IS4 - IS6	
	domains, of PMD-R strain with that of the susceptible	
	strains, PMD, Liverpool and China strains	

xviii

LIST OF FIGURES (continued)

Figure

Page

67

68

3.9 Alignment of the amino acid sequences of the *Ae*. *aegypti* voltage-gated sodium channel protein, IIS1-IIS6 domains, of PMD-R strain with that of the susceptible strains, PMD, Liverpool and China strains

3.10 Alignment of the amino acid sequences of the Ae. *aegypti* voltage-gated sodium channel protein,
IIIS4-IVS2 domains, of PMD-R strain with that of
the susceptible strains, PMD, Liverpool and China
strains

3.11 Alignment of the sodium channel amino acid sequence of the Ae. aegypti Liverpool strain with that of M. domestica housefly Vssc1

3.12 Alignment of voltage-gated sodium channel sequences from *Ae. aegypti* PMD-R strain with that from the other arthropods and non-arthropods in the region of IIIS6 domain

3.13 The log time- probit mortality line of the parental strains, the F_1 hybrids and the F_2 backcrosses after exposure to 0.75% permethrin paper

69

72

LIST OF FIGURES (continued)

Figure		Page
3.14	The log concentration-probit mortality lines (A) and log	76
	concentration-mortality response curves (B) of the	
	parental, F1 hybrids and backcrossed individuals after	
	exposure to permethrin	
3.15	The log concentration-probit mortality lines of the	79
	parental strains, F1 progeny after exposure to	
	permethrin compare to those of the F1 hybrids after	
	exposure to permethrin with the PBO synergist	
3.16	Detection of the F1534C genotyping using TaqMan	81
	SNP genotyping assay	
3.17	Scatter plot of end point fluorescence intensities using	82
	the TaqMan SNP genotyping assay	
3.18	Effect of sample dilution on fluorescence intensity	83
3.19	Characteristic agarose gel and sensitivity test for the	85
	AS-PCR assay for the detection of the F1534C mutation	
3.20	Estimated frequency distributions of the homozygous	91
	wild type (F/F1534), heterozygous (F/C1534) and	
	homozygous mutant (C/C1534) genotypes in Ae.	
	aegypti in Thailand	

LIST OF FIGURES (continued)



ลิขสิทธิ์มหาวิทยาลัยเชียงไหม Copyright[©] by Chiang Mai University All rights reserved

ABBREVIATIONS

AS-PCR	Allele Specific PCR
ADH	Alcohol dehydrogenase
ALDH	Aldehyde dehydrogenase
bp	base pair
BNPP	(Bis (4-nitrophenyl)-phosphate)
cDNA	Complementary DNA
CI	Confidence interval
DDT	Dichlorodiphenyltrichloroethane
DHF	Dengue heamorhagic fever
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide triphosphates
DSC1	Drosophila sodium channel 1
FRET	Fluorescence resonance energy transfer
GST	Glutathione-S-transferase
GABA	Gamma-Aminobutyric
HOLA	Hot oligonucleotide ligation assay
HRM	High resolution melts analysis
kDa S	Kilodalton Meserve O
kdr	Knockdown resistance
LC	Lethal concentration
LT	Lethal time

xxi

MGB	Minor groove binder
NFQ	Nonfluorescent quencher
NTC	No template control
P 998	Probability
para	Paralytic-temperature sensitive sodium channel
РВО	Piperonyl butoxide
PCR	Polymerase chain reaction
R _n	Normalized reporter
RR	Resistance ratio
SE	Standard error
SNP	Single nucleotide polymorphism
SEARO	South East Asia Regional Office
SSOP-ELISA	Sequence specific oligonucleotide probe enzyme-
	linked immunosorbent assay
RNA	Ribonucleic acid
TipE	Temperature-induced paralysis, locus E
T _m	Melting temperature
UV	Ultraviolet
Vssc1	Voltage sensitive sodium channel
WHO	World health organization

xxii