

## TABLE OF CONTENTS

	PAGE
ACKNOWLEDGEMENT	iii
ABSTRACT	iv
LIST OF TABLES	xi
LIST OF ILLUSTRATIONS	xiii
ABBREVIATIONS	xiv
I. INTRODUCTION	1
II. LITERATURE REVIEW	
A. Dengue virus	4
1. Structure and composition of virion	4
2. Genome structure and viral proteins	4
3. Envelope glycoprotein	6
4. NS1, NS3 and NS5 proteins	7
5. Heterogeneity of dengue viruses	10
5.1. Serologic study	11
5.2. RNA fingerprinting study	12
5.3. cDNA-RNA hybridization study	12
5.4. Nucleotide sequence comparison	13
6. Clinical features	13
6.1. Undifferentiated fever	13
6.2. Dengue fever	13
6.3. Dengue hemorrhagic fever	14

B. Epidemiology	15
1. Global level	15
2. Asia	16
3. Molecular epidemiology	18
3.1. Dengue serotype 1 virus	18
3.2. Dengue serotype 2 virus	19
3.3. Dengue serotype 3 virus	25
3.4. Dengue serotype 4 virus	25
III. MATERIALS AND METHODS	
1. Dengue viruses	26
2. Oligonucleotide primer for RT-PCR amplification	26
3. Preparation of genomic RNA	35
4. Synthesis of complementary DNA	36
5. Amplification of dengue envelope gene	36
6. Oligonucleotides and labeling	37
7. Dot blot hybridization	39
8. Densitometric analysis of hybridization signal	42
9. Determination of sensitivity and specificity	44
10. Nucleotide sequence analysis	45
IV. RESULTS	
1. Conservation of differentiating nucleotide substitutions	47
2. Sensitivity and specificity of oligonucleotide probes	59
3. Predominance of subtype IIIa among dengue serotype 2 viruses in 1994	73
4. Descent of subtype IIIa viruses	75

V. DISCUSSION	80
VI. SUMMARY	95
REFERENCES	98
APPENDIX	
Appendix A. Probe 141-IIIa and 141-IIIb	110
Appendix B. Probe 203-IIIa and 203-IIIb	113
Appendix C. Probe 308-IIIa and 308-IIIb	116
Appendix D. Probe 484-IIIa and 484-IIIb	119
Appendix E. Probe 491-IIIa and 491-IIIb	122
Appendix F. Probe 346-IIIa-87	125
Appendix G. Reagents and instruments	128
CURRICULUM VITAE	132

## LIST OF TABLES

Table	Page
1 The genotypic comparison of dengue serotype 2 viruses from various studies	22
2 Dengue viruses derived from the 1994 epidemic season in Bangkok	27
3 Characteristics of dengue virus isolates derived from the 1994 epidemic season in Bangkok	29
4 Dengue virus isolates derived before the 1994 epidemic season	31
5 Oligodeoxyribonucleotide primers for reverse transcription-polymerase chain reaction and nucleotide sequence analysis	34
6 Oligodeoxyribonucleotide probes for differential hybridization	38
7 Ratio of adjusted volume between various probes and probe C1204	70
8 Ratio of maximum optical density between various probes and probe C1204	71
9 Sensitivity and specificity of various probes as determined by using the lowest adjusted volume ratio value as the cutoff point	72
10 Sensitivity and specificity of various probes as determined by using the lowest maximum optical density ratio value as the cutoff point	74

Table	Page
11 Reactivity of dengue serotype 2 viruses derived from the 1994 epidemic season with various probes using the lowest ratio value of various probes as the cutoff point	76
12 Ratio of adjusted volume and maximum optical density between probe 346-IIIa-87 and probe C1763	78
13 Sensitivity and specificity of probe 346-IIIa-87 as determined by using the highest adjusted volume ratio and maximum optical density ratio values of non-subtype IIIa strains (1987 epidemic season) as the cutoff point	79

## LIST OF ILLUSTRATIONS

Figure		Page
1	Dot spotting of PCR products on nylon membrane	40
2	Nucleotide sequence of the envelope gene of D94-039, D94-179, D94-376, D94-548 and other dengue type 2 virus isolates classified as subtype IIIa or IIIb	48
3	Deduced amino acid sequence of envelope protein of D94-039, D94-179, D94-376, D94-548 and other dengue type 2 virus isolates classified as subtype IIIa or IIIb	60
4	Number of nucleotide differences and amino acid differences of envelope gene/protein among selected dengue serotype 2 viruses isolated from 1980, 1987 and 1994 epidemic seasons	64
5	An example of the hybridization signal when reacted with probe C1204	66
6	An example of the hybridization signal when reacted with probe 141-IIIa	67
7	An example of the hybridization signal when reacted with probe 141-IIIb	68
8	An example of the hybridization signal when reacted with probe 346-IIIa-87	69

## ABBREVIATIONS

A	Adenine
C	Cytosine
°C	Degree celsius
cDNA	Complementary DNA
CDP-star	Disodium 2-chloro-5-(4-methoxyspiro{ 1,2-dioxetane-3,2'-(5'-chloro)tricyclo[3.3.1.1 <sup>3,7</sup> ]decan}-4-yl)-1-phenyl phosphate
DF	Dengue fever
DHF	Dengue hemorrhagic fever
DIG-ddUTP	Digoxigenin dideoxyuridine-triphosphate
dNTP	Deoxyribonucleoside triphosphate
DEPC	Diethylpyrocarbonate
DSS	Dengue shock syndrome
DTT	Dithiothreitol
E	Envelope
EDTA	Ethylenediaminetetraacetic acid
G	Guanine
h	Hour
kb	Kilobase
kDa	Kilodalton
M	Membrane
MgCl <sub>2</sub>	Magnesium chloride
min	Minute
μl	Microliter

mM	Millimolar
NaCl	Sodium chloride
NaOH	Sodium hydroxide
nm	Nanometer
NS	Nonstructural
O.D.	Optical density
PCR	Polymerase chain reaction
pmol	Picomole
pre-M	Premembrane
PVP	Polyvinylpyrrolidone
rpm	Round per minute
RT-PCR	Reverse transcription-polymerase chain reaction
SD	Standard deviation
SDS	Sodium dodecyl sulfate
T	Thymine
TAE	Tris/acetate/EDTA
T <sub>m</sub>	Melting temperature
TMAC	Tetramethylammonium chloride