

TABLE OF CONTENTS

	PAGE
ACKNOWLEDGEMENTS	III
ABSTRACT	IV
TABLE OF CONTENTS	VIII
LIST OF FIGURES	XI
LIST OF TABLES	XIII
ABBREVIATION	XIV
 CHAPTER 1 INTRODUCTION	 1
1.1 Statement and significance of the problem	1
1.2 Literature review	3
1.2.1 Hemoglobin structure and function	3
1.2.2 Genetic control and synthesis of normal hemoglobin	4
1.2.3 Globin gene cluster	6
1.2.4 The thalassemia	9
1.2.5 The α -thalassemias and it molecular defects	9
1.2.5.1 α^0 - thalassemia or α - thalassemia 1	10
1.2.5.2 α^+ - thalassemia or α - thalassemia 2	11
1.2.5.3 Nondeletion α - thalassemia	15
1.2.6 The clinical syndromes of α -thalassemia	17
1.2.6.1 Silent carrier : the lost of a single α - globin genes	17
1.2.6.2 Alpha- thalassemia trait : the lost of two α - globin genes	18
1.2.6.3 Hb H disease	18
1.2.6.4 Hb Bart's hydrops fetalis	20
1.3 The molecular approaches in detection of α - thalassemia 2 ($-\alpha^{3.7}$)	21
1.3.1 PCR technology	21

IX

1.3.2 General principles	21
1.3.3 The standard reaction	23
1.4 Objectives	27
 CHAPTER 2 RESEARCH DESIGN AND METHODS	 28
2.1 Research design for detection of α -thalassemia2: rightward type ($-\alpha^{3.7}$)	28
2.2 Methods	30
2.2.1 Samples	30
2.2.2 Genomic DNA preparation	30
2.2.3 Polymerase chain reaction	33
2.2.3.1 Primer selection	33
2.2.3.2 Components of polymerase chain reaction	37
2.2.4 Agarose gel electrophoresis	39
2.2.5 Hemoglobin electrophoresis (Hemoglobin typing)	40
2.2.6 Hematological examination	42
2.2.6.1 Hematocrit by microhematocrit method	42
2.2.6.2 Red blood cell count	44
2.2.6.3 Hemoglobinometry by cyanmethemoglobin method	47
2.2.6.4 Osmotic fragility test	49
2.2.6.5 Acid elution test	51
2.2.6.6 Inclusion bodies test	53
 CHAPTER 3 RESULTS	 55
3.1 Genomic DNA preparation	55
3.2 Polymerase chain reaction	55
3.2.1 Optimization of the PCR component	55

3.2.2 The identification of $-\alpha^{3.7}$ genotype by PCR technique	59
3.2.3 The reliability of the PCR protocol	61
3.3.4 Detection of $-\alpha^{3.7}$ deletion in two HB H disease family	61
3.2.5 The identification of $-\alpha^{3.7}$ genotype in Northern Thai population by PCR technique	67
3.3 Results from hematological screening	72
3.3.1 The hematological characteristic of α -thalassemia ² carriers ($-\alpha^{3.7}$)	72
3.3.2 Result from hemoglobin electrophoresis	73
3.3.3 Result from inclusion bodies test	75
3.3.4 Result from acid elution test	76
CHAPTER 4 DISCUSSION	77
CHAPTER 5 CONCLUSION	81
REFERENCES	82
APPENDIX	91
VITA	98

LIST OF FIGURES

FIGURE	PAGE
1. Hemoglobin structure	3
2. Schematic representation of the α - globin and β - globin gene cluster	5
3. Schematic representation of the α - globin gene cluster	7
4. Schematic representation of the β - globin gene cluster	8
5. The deletion of the alphaglobin gene cluster that are responsible for α^0 and thalassemia.	13
6. The single alpha globin gene deletion and its proposed mechanisms.	14
7. Schematic representation of amplification of target DNA sequence by polymerase chain reaction technique	22
8. Schematic representation of the procedure in this research	29
9. Schematic representation of genomic DNA preparation	32
10. The locations of amplification for the specific primers	33
11. Hemocytometer	46
12. Manner of counting erythrocytes in one of small squares	46
13. Hemoglobin standard calibration curve	48
14. The optimization of the deoxynucleotide 5'-triphosphates concentration for detection of $-\alpha^{3.7}$ by PCR	56
15. The optimization of annealing temperature for detection of $-\alpha^{3.7}$ kb deletion by PCR	57
16. The optimization of extension time for detection of $-\alpha^{3.7}$ kb deletion by PCR	58
17. Identification of $-\alpha^{3.7}$ kb deletion in genomic DNA samples by PCR	60

XII

18. Detection of $-\alpha^{3.7}$ kb deletion in the normal DNA samples by PCR	62
19. Detection of $-\alpha^{3.7}$ kb deletion in the normal and positive control genomic DNA by PCR (N1, P1-4, and H1)	63
20. Detection of $-\alpha^{3.7}$ kb deletion in the normal and positive control genomic DNA by PCR (N1-2, P5-7, and H2)	64
21. Detection of $-\alpha^{3.7}$ kb deletion in hemoglobin H family (1st) by PCR	65
22. Detection of $-\alpha^{3.7}$ kb deletion in hemoglobin H family (2nd) by PCR	66
23. Detection of $-\alpha^{3.7}$ kb deletion in genomic DNA samples by PCR (Samples 12-18)	68
24. Detection of $-\alpha^{3.7}$ kb deletion in genomic DNA samples by PCR (Samples 48-56)	69
25. Detection of $-\alpha^{3.7}$ kb deletion in genomic DNA samples by PCR (Samples 191-196)	70
26. Detection of $-\alpha^{3.7}$ kb deletion in genomic DNA samples by PCR (Samples 292-301)	71
27. The result from hemoglobin electrophoresis (hemoglobin typing)	74
28. The red cell inclusion bodies (2 +) in peripheral blood of hemoglobin H disease subject ($--/\alpha^{3.7}$)	75
29. Acid elution preparation of blood film shows hemoglobin F (4+) from cord blood sample.	76

LIST OF TABLES

TABLES	PAGE
1. Nondeletional mutants that cause α - thalassemia	16
2. Dilutions and O.D. for hemoglobin standard curve	48
3. The results from the detection of alpha 3.7 kb deletion in Northern Thai population by PCR technique	67
4. The hematological data of the 34 blood samples of α - thalassemia 2 ($-\alpha^{3.7}$) carriers	72
5. Relative frequencies of hemoglobin types in 400 blood samples	73

ABBREVIATIONS

A	adenine
A°	angstrom
bp	base pair
C	cytosine
°C	degree celsius
cm	centimetre
dl	decilitre
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide triphosphate
G	guanine
Hb	hemoglobin
HVR	hyper variable region
kb	kilobase
LCR	locus control region
L	litre
mA	milliampere
MCV	mean corpuscular volume
min	minute
ml	millilitre
mM	millimolar
µg	microgram
µl	microlitre
µM	micromolar
MW	molecular weight
nm	nanometre
Hct	hematocrit
OD	optical density

OF	osmotic fragility
PCR	polymerase chain reaction
rpm	revolution per minute
RBC	red blood cell
sec	second
T	thymine
T _m	melting temperature
U	unit
UTR	untranslated region
UV	ultraviolet
V	voltage