

TABLE OF CONTENTS

	page
ACKNOWLEDGEMENT	iii
ABSTRACT	v
TABLE OF CONTENTS	xi
LIST OF FIGURES	xiv
LIST OF TABLES	xv
ABBREVIATION	xvi
CHAPTER I INTRODUCTION	1
1.1 Statement and significance of the problem	1
1.2 Literature review	3
1.2.1 Hemoglobin function	3
1.2.2 Hemoglobin production in human	4
1.2.3 The α -globin gene expression	6
1.2.4 Molecular defects in α - thalassemia	8
1.2.4.1 Gene deletion α -thalassemia	8
1.2.4.2 Non-gene deletion α -thalassemia	9
1.2.5 Deletion of a single α -globin gene	12
1.2.6 Deletion of both α -globin genes	13
1.2.7 Hemoglobin H disease	14
1.2.8 Hemoglobin Bart's hydrops fetalis syndrome	16
1.3 Principle and Rationale	18

1.3.1 Introduction to polymerase chain reaction	18
1.3.2 Principle of the PCR method	19
1.3.3 Standard method of PCR	21
1.3.4 PCR optimization	22
1.3.5 Principle and application of erythrocyte osmotic fragility test	24
1.3.6 Rationale	25
1.4 Objectives of this study	28
CHAPTER II RESEARCH DESIGNS AND METHODS	29
2.1 Research designs	29
2.2 Methods	31
2.2.1 Blood samples	31
2.2.2 Hb A ₂ determination	31
2.2.3 Erythrocyte osmotic fragility test	34
2.2.4 Genomic DNA preparation	35
2.2.5 Polymerase chain reaction	36
2.2.5.1 Components of the PCR	36
2.2.5.2 PCR procedure	38
2.2.5.3 Agarose gel electrophoresis	40
2.2.5.4 Photography	41
CHAPTER III RESULTS	42
3.1 Hb A ₂ determination	42

3.2 Erythrocyte osmotic fragility test	45
3.3 Polymerase chain reaction	48
3.4 The correlation of PCR and EOFT with Hb A ₂	51
determination for diagnostic value	
CHAPTER IV DISCUSSION AND CONCLUSION	53
REFERENCES	59
VITA	70

LIST OF FIGURES

Figure	Page
1 Organization of the human globin genes and hemoglobin production in each stage of human development	5
2 The major deletion in α -thalassemia 2	13
3 The major deletion in α -thalassemia 1	14
4 Schematic diagram of the PCR process	20
5 Micro-column chromatography equipment	33
6 The position of primers for PCR to detect α -thalassemia1 (Southeast Asia type)	37
7 The quality control of HbA ₂ determination	44
8 The kinetic of hemolysis of normal person and α -thalassemia 1 trait	46
9 The quality control of erythrocyte osmotic fragility test	47
10 Detection of α -thalassemia-1 SEA type by PCR	49
11 Amplification of the DNA from the first 10 women using primer A and C	50
12 Erythrocyte osmotic fragility value of non-traits and α -thalassemia 1 traits	52

LIST OF TABLE

Table	Page
1 Features of some gene deletion causing α -thalassemia	10
2 Non-gene deletion forms of α -thalassemia	11
3 Frequencies of thalassemias and abnormal hemoglobins in different regions of Thailand	17
4 The HbA ₂ levels of the 500 analyzed pregnant women	43
5 Erythrocyte osmotic fragility values of normal women and of α -thalassemia 1 traits that have HbA ₂ levels lower than 4%	51

ABBREVIATIONS

bp	base pair
Hb	Hemoglobin
kb	kilobase
No.	number
OD	optical density
BSA	bovine serum albumin
DNA	deoxyribonucleic acid
PCR	polymerase chain reaction
rpm	revolution per minute
dNTP	Deoxynucleotide triphosphates
UV	Ultraviolet