

THEBLE OF CONTENTS

	PAGE
ACKNOWLEDGEMENT	iii
ENGLISH ABSTRACT	iv
THAI ABSTRACT	vi
TABLE OF CONTENTS	vii
LIST OF TABLES	x
LIST OF FIGURES	xi
ABBREVIATIONS	xiii
CHAPTER I INTRODUCTION	1
1.1 Statement and function of the problem	1
1.2 Objective of this study	2
CHAPTER II LITERATURE REVIEWS	3
2.1 Structure and function of hemoglobin	3
2.2 Types of hemoglobin	3
2.3 The localization and organization of globin gene	5
2.3.1 The α -globin gene cluster	5
2.3.2 The β -globin gene cluster	6
2.4 The β -thalassemia	8
2.4.1 Gene deletion	10
2.4.2 Non-deletion	10
2.5 Sickle / β -thalassemia	13
2.6 The polymerase chain reaction (PCR)	15
2.7 The thermal cycle sequencing	17

2.8 Thermal cycle sequencing with dRhodamine dye terminators and AmpliTaq® DNA polymerase, FS enzymes	17
2.9 ABI PRISM 310 Genetic Analyzer	18
CHAPTER III RESEARCH DESIGN AND METHODS	20
Research designs	20
Chemical and materials	20
Case History	20
1. Laboratory studies	22
Genomics DNA extraction from whole blood	22
1.1 Amplification of the β -globin gene exons by PCR	22
1.2 Chain-termination cycle sequencing	28
Purification of the amplicons	28
Sequencing by chain-termination cycle sequencing technique	29
Cycle sequencing reaction mixture	29
A) Dye terminator premix	29
B) Sequencing primer	29
Chain-termination cycle sequencing technique	30
Ethanol purification of sequencing extension products	31
Preparing and loading the samples on to the sequencer	31
CHAPTER IV RESULTS	32
1. Laboratory studies	32
1.1 Amplification of the β -globin gene exons by PCR	32

1.2 Chain-termination cycle sequencing	
Confirmation of single base substitution at splice junction in exon 1 of β -globin gene by using chain termination cycle sequencing of patient' s mother	36
CHAPTER V DISCUSSION AND CONCLUSION	38
REFERENCES	41
APPENDIX	47
Appendix A. List of the chemicals and materials used in this study	47
Appendix B. List of instruments used in this study	50
Appendix C. Reagent preparations	51
VITA	54

LIST OF TABLES

TABLE	PAGE
1 The normal human hemoglobins and their globin subunits	5
2 Molecular mechanism of β -thalassemia in Thailand	12
3 Primer used to amplify the β -globin gene exons	32
4 DNA contraction of each exons of the β -globin gene	29
5 Cycle sequencing reaction mixture	30
6 Three-step PCR protocol used for cycle sequencing	30

LIST OF FIGURES

FIGURE	PAGE
1 HPLC elution profile for Hb S using the Bio-Rad Variant™	1
2 Cellulose acetate electrophoresis of blood hemolysates from the patient 's family	2
3 Cellulose acetate electrophoresis of blood hemolysates from the patient 's family	2
4 Secondary structure of the human hemoglobin β -globin subunit	4
5 The location of β -globin gene cluster	7
6 Linkage map of the human β -globin gene cluster	7
7 The general structure and organization of the globin genes	8
8 Inheritance of β -thalassemia	9
9 The mutation of splice junction	13
10 Inheritance of hemoglobin genes from parents with sickle cell trait and thalassemia trait	14
11 The polymerase chain reaction	16
12 Flow chart of the research design in this study	21
13 Flow chart of the DNA extraction by the Chelex method	23
14 The position of the primers	24
15 The effect of glycerol concentrations in the amplification of exon 1	32
16 The effect of glycerol concentrations in the amplification of exon 2	33

17 The effect of glycerol concentrations in the amplification of exon 3	33
18 Sequence analysis of PCR amplified DNA of exon 1 of the β -globin gene of patient	34
19 Sequence analysis of PCR amplified DNA of exon 1 of the β -globin gene of patient	35
20 Sequence analysis of PCR amplified DNA of exon 1 of the β -globin gene of patient' s mother	36
21 Pedigree diagram of the patient and family members	37

ABBREVIATIONS

The abbreviations and symbols used throughout this thesis

bp	base pair
BSA	bovine serum albumin
dATP	deoxyadenosine triphosphates
dCTP	deoxycytidine triphosphates
ddNTPs	dideoxynucleotides triphosphates
dGTP	deoxyguanosine triphosphates
DNA	deoxynucleic acid
dNTPs	deoxynucleotides triphosphates
dTTP	deoxythymidine triphosphates
EDTA	ethylene diamine tetraacetate
Hb	hemoglobin
HPLC	High Performance Liquid Chromatography
nt	nucleotides
OPD	out patient department
OD	optical density
PCR	polymerase chain reaction
q.s	quantity of sample
rpm	revolution per minute
<i>Taq</i> DNA polymerase	<i>Thermus aquaticus</i> DNA polymerase
Tris	Trishydroxymethyl aminomethane
Tris-HCl	Trishydroxymethyl aminomethane titrated with HCl