TABLE OF CONTENTS

	Page
APPROVAL PAGE	ii
ACKNOWLEDGEMENTS	iii
ABSTRACT	iv
LIST OF TABLES	X
LIST OF FIGURES	xi
ABBREVIATIONS AND SYMBOLS	xiii
CHAPTER I: INTRODUCTION	1
CHAPTER II: LITERATURE REVIEWS	6
1. Infertility	6
2. Causes of male infertility	7
3. The endocrine control of spermatogenesis	11
4. Y chromosome and Y chromosome microdeletions	13
5. Mechanism of Y chromosome microdeletions	14
6. Prevalence of Y chromosome microdeletions	16
7. Y chromosome microdeletions and infertility	18
8. Genotype-phenotype correlation	18
9. Clinical significance of Y chromosome microdeletions	19
10. Method of Y chromosome microdeletion detection	20
11. Multiplex PCR	21
12. Development of multiplex PCR	23
CHAPTER III: RESEARCH DESIGN, MATERIALS AND METHODS	30
1. Research design	30
2. Materials and methods	32
2.1 Subjects	32

		2.2 Oligonucleotide primers	34	
		2.3 Specimen collection and preparation	34	
		2.4 Multiplex PCR	37	
		2.5 Detection of Multiplex PCR products	42	
		2.6 Quantification of serum hormone levels	42	
		2.7 Cytogenetic evaluation	44	
		2.8 Data analysis	44	
CHAPTER IV: RESULTS				
		1. Study subjects	45	
		2. Genomic DNA concentration	48	
		3. Determination of each specific primer efficiency	48	
		4. Optimization of multiplex PCR conditions	55	
		5. Prevalence of Y chromosome microdeletions in infertile males	65	
		6. Cost comparison between the In-house multiplex PCR assay	69	
and commercial kit (Promega®)				
CHAPTER V: DISCUSSION AND CONCLUSION 7				
REFERENCES 7			76	
	APPENDICES			
	CIRRICURU	UM VITAE	95	

ลิปสิทธิ์มหาวิทยาลัยเชียงใหม่ Copyright © by Chiang Mai University All rights reserved

LIST OF TABLES

Table		Page
2.1	The prevalence of Y chromosome microdeletions in different parts	17
	of the world	
3.1	The sequences and product sizes of all 12 primers used in this study	36
3.2	The details of the four multiplexes in this study	39
3.3	AZF marker genes in this study	42
4.1	Classification of patients according to the sperm concentration	45
4.2	Clinical characteristics of the patients	46
4.3	Hormonal profiles of azoospermic and oligospermic males with	47
	Y chromosome microdeletions	
4.4	Chromosome anomalies in azoospermic and oligospermic males	48
4.5	The final concentrations of primers used in multiplex PCR	66
4.6	Summary of patients with regions of Y chromosome microdeletions	70
4.7	Comparison of Cost between In-house multiplex PCR and Promaga®kit	71

ລິ**ປສິກລິ້ມหາວົກຍາລັຍເຮີຍວໃหມ່** Copyright © by Chiang Mai University All rights reserved

х

LIST OF FIGURES

Figure		Page
2.1	Schematic representation of the Y chromosome	13
2.2	Schematic representation of the AZF locus in Yq11	15
3.1	The schematic diagram of the research design in this study	33
3.2	The location of all 12 AZF-marker genes on the human Y chromosome	39
4.1	The occupations of infertile males who are azoospermia or oligospermia	46
4.2	The amplification products of singleplex PCR in multiplex set # 1	49
4.3	Singleplex PCR amplification product (800 bp) of <i>RBM1</i> gene at	50
	various annealing temperatures	
4.4	The single PCR amplifications of <i>RBM1</i> gene achieved when different	50
	primer concentrations were used	
4.5	Amplification products of multiplex PCR set # 1.	51
4.6	The PCR products of singleplex PCR amplification and PCR products	52
	of four primer pairs in multiplex PCR set # 2	
4.7	The PCR products of singleplex PCR amplification and four PCR	53
	products of multiplex PCR set # 2 with both PCR product of DAZ(sY28.	3)
4.8	PCR products of three single-primer PCR and one multiplex PCR(set #3	3) 54
4.9	PCR products of four single-primer and one multiplex PCR(set # 4)	54
4.10	Multiplex PCR products at different concentrations of EIF1AY primers	55
4.11	Optimized multiplex PCR set # 1, showing all amplification products	56
4.12	Amplification products of multiplex PCR set # 2 at different	57
	concentrations of DAZ(sY283) primers	
4.13	Products of multiplex PCR set # 3	58
4.14	PCR products generated by the multiplex PCR set # 4, when 2	58
	concentrations of TTY2 primers were used	
4.15	Products of multiplex PCR set # 4 after optimization	59

4.16	PCR products of multiplex PCR set # 2, using various concentrations	60
	of genomic DNA	
4.17	PCR products of multiplex PCR set # 4, with various concentration	60
	of genomic DNA	
4.18	Amplification products of multiplex PCR set # 2 with an extension time	61
	of 1.0 and 1.5 min	
4.19	PCR products of multiplex PCR set # 4, with an extension time of 1.0	62
	and 1.5 min	
4.20	Amplification products of multiplex PCR # 1, with 35 and 40 PCR	63
	cycles	
4.21	Amplification products of multiplex PCR # 4, with 35 and 40 PCR	64
	cycles	
4.22	Amplification products of multiplex PCR set # 4 with different	64
	extension times	
4.23	Amplification products of the 4 optimized multiplex PCR sets	67
4.24	Detection of Y chromosome microdeletions using the four optimized	68
	multiplex PCRs	
4.25	Schematic diagram of Y chromosome microdeletions in the six infertile	70
	males in this study	

âðânອົ່ມກາວົກຍາລັຍເຮີຍວໃກມ Copyright © by Chiang Mai University All rights reserved

ABBREVIATIONS AND SYMBOLS

AZF	Azoospermia factor
%	percent
Tag	Thermus aquaticus
DNA	deoxyribonucleic acid
mM	millimolar
μM	micromolar
μΙ	microliter
°C	degree Celsius
bp	base pair
dNTPs	deoxyribonucleotide triphosphate
dATP	deoxyadenosine triphosphate
dCTP	deoxycytidine triphosphate
dGTP	deoxyguanosine triphosphate
dTTP	deoxythymidine triphosphate
EIA	enzyme immunoassay
g J IIII	gram or gravity
М	molarity
mg	milligram
Kb	kilobase
Mb	megabase
MgCl ₂	magnesium chloride
mlby Chia	millititer all University
min	minute
N	normality
ng	nanogram
nm	nanometer
OD	optical density

xiii

PBMC	peripheral blood mononuclear cells
PCR	polymerase chain reaction
RNA	ribonucleic acid
rpm	round per minute
UDDD	unit
DFFRY	ubiquitin specific protease 9, Y chromosome
DBY	DEAD (Asp-Glu-Ala-Asp) box
	polypeptide 3, Y-linked
EIFIAY	eukaryotic translation initiation factor 1A,
	Y chromosome
PRY	testis-specific PTP-BL-related protein on
	Y chrmosome
TTY2	testis-specific testis transcript Y 2
RBM1	RNA binding motif protein 1
DAZ	deleted in azoospermia
BPY2	basic protein on Y chromosome 2
CDY1	chromodomain protein, Y-linked, 1
SMCY	Smcy homolog, Y-linked (mouse)
SRY	sex determining region Y

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่ Copyright © by Chiang Mai University All rights reserved