GENETIC DIVERSITY OF THAI INDIGENOUS PIGS, WILD BOARS AND CHINESE QIANBEI BLACK PIGS BASED ON MICROSATELLITE DNA AND SEQUENCE POLYMORPHISM OF MITOCHONDRIA DNA *CYTOCHROME* b GENE

Yang Shenglin

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Animal Production Technology

Suranaree University of Technology

Academic Year 2007

ความหลากหลายทางพันธุกรรมของสุกรพื้นเมืองไทย สุกรป่า และสุกรจีนพันธุ์เชียนเปแบลค โดยการใช้ข้อมูลไมโครแซทเทลไลท์ดีเอ็นเอ และการเปรียบเทียบลำดับเบสในส่วนของยีน cytochrome b.

นาย หยาง เซิง หลิน

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
สาขาวิชาเทคโนโลยีการผลิตสัตว์
มหาวิทยาลัยเทคโนโลยีสุรนารี
ปีการศึกษา 2550

GENETIC DIVERSITY OF THAI INDIGENOUS PIGS, WILD BOARS AND CHINESE QIANBEI BLACK PIGS BASED ON MICROSATELLITE DNA AND SEQUENCE POLYMORPHISM OF MITOCHONDRIA DNA *CYTOCHROME* b GENE

Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy

Thesis Examining Committee

_____
(Asst. Prof. Dr. Pramote Peangkoum)
Chairperson

_____
(Assoc. Prof. Dr. Pongchan Na-Lampang)
Member (Thesis Advisor)

_____
(Dr. Surintorn Boonanuntanasarn)
Member

_____
(Prof. Dr. Uthairat Na-Nakorn)
Member

_____
(Dr. Pakanit Kupittayanant)
Member

_____
(Prof. Dr. Pairote Sattayatham)
Vice Rector for Academic Affairs

_____
(Asst. Prof. Dr. Suwayd Ningsanond)
Dean of Institute of Agricultural Technology

หยาง เซิง หลิน : ความหลากหลายทางพันธุกรรมของสุกรพื้นเมืองไทย สุกรป่า และสุกรจีนพันธุ์เชียนเปแบลค โดยการใช้ข้อมูลไมโครแซทเทลไลท์ดีเอ็นเอ และการเปรียบเทียบลำดับเบสในส่วนของยีน cytochrome b. (GENETIC DIVERSITY OF THAI INDIGENOUS PIGS, WILD BOARS AND CHINESE QIANBEI BLACK PIGS BASED ON MICROSATELLITE DNA AND SEQUENCE POLYMORPHISM OF MITOCHONDRIA DNA *CYTOCHROME* b GENE) อาจารย์ที่ปรึกษา : รองศาสตราจารย์ ดร.พงษ์ชาญ ณ ลำปาง, 162 หน้า.

จำนวนสุกรพันธุ์พื้นเมืองไทยลดลงอย่างรวดเร็วตั้งแต่เมื่อเริ่มมีการนำสุกรพันธุ์ต่างประเทศเข้ามาเพื่อปรับปรุงพันธุ์สุกรในช่วง ค.ศ. 1960 ตราบจนถึงปัจจุบันเป็นที่ทราบกันน้อยเกี่ยวกับความผันแปรทางพันธุกรรมทั้งในอดีตและปัจจุบันของสุกรพื้นเมืองไทยที่มีฐานการศึกษาในระดับโมเลกุล ดังนั้น วัตถุประสงค์ของการศึกษานี้จึงเพื่อศึกษาความหลากหลายทางพันธุกรรมระหว่างสุกรพื้นเมืองไทยสองประชากร (สุกรไทยภาคใต้ (ST) และสุกรไทยภาคตะวันออกเฉียงเหนือ(NT)) สุกรป่า (WB) และสุกรจีนพันธุ์เชียนเปแบลค (CQB) โดยใช้ฐานการศึกษาตัวบ่งชี้ไมโครแซทเทลไลท์ และเพื่อศึกษาเปรียบเทียบลำดับเบสของยีนไซโตโครมบี ในระหว่างสุกรทั้งสี่ประชากรนี้ และได้ศึกษาความสัมพันธ์ทางพันธุกรรมระหว่างสุกรทั้งสี่ประชากรนี้กับสุกรพันธุ์ต่างประเทศโดยการเปรียบเทียบลำดับเบสของยีนไซโตโครมบีด้วย ในเบื้องต้นได้ทำการทดลองเพื่อหาเนื้อเยื่อที่เหมาะสมในการนำมาสกัดจีโนมิสดีเอ็นเอ (genomic DNA) คือ จากตัวอย่างเลือดและปมรากขน สำหรับนำมาใช้ในปฏิกิริยาพีซีอาร์ของไมโครแซทเทลไลท์ที่ตำแหน่ง S0225 และ S0227 และของยีนไซโตโครม บีในไมโตคอนเดรีย ผลการทดลองชี้ว่าดีเอ็นเอที่ได้จากตัวอย่างเลือดและปมรากขนสามารถใช้เป็นเทมเพลทสำหรับการทำพีซีอาร์ไมโครแซทเทลไลท์ และยีนไซโตโครมบี ได้ ดังนั้นการศึกษาครั้งนี้จึงจะใช้การเก็บตัวอย่างดีเอ็นเอจากปมรากขน เพราะเป็นวิธีการที่ง่ายและไม่ทำให้สุกรบาดเจ็บ จากนั้นได้ทำการวิจัยหลักเพื่อประเมินค่าความผันแปรทางพันธุกรรมของสุกรพันธุ์ไทยทั้งสองประชากรโดยการใช้ไมโครแซทเทลไลท์ไพรเมอร์จำนวน 12 ไพรเมอร์ ผลการศึกษาพบว่าประชากรของสุกรไทยภาคใต้และสุกรไทยภาคตะวันออกเฉียงเหนือแสดงความเป็นเฮตเตอโรไซโกตเฉลี่ย (HE = 0.86 และ 0.84) และค่าพีไอซี (PIC; Polymorphism Information Content) (0.82 และ 0.81) ซึ่งสูงกว่าสุกรพันธุ์ยุโรปและสุกรพันธุ์จีนบางพันธุ์ และพบว่าประชากรที่ศึกษาทั้งสี่ประชากรอยู่ในสมดุลย์ฮาร์ดี-วายเบิร์ก การวิเคราะห์สายสัมพันธ์ UPGMA ที่อาศัยฐานระยะห่างทางพันธุกรรมตามมาตรฐาน Nei's D_A แสดงว่าสุกรพันธุ์จีนและสุกรพันธุ์ไทยทั้งสอง

ประชากรรวมกันอยู่ในสาขาเดียวกันโดยมีค่า bootstrap 100% แต่สุกรป่ารวมอยู่ในอีกสาขาหนึ่ง จึงอนุมาณว่าสุกรพื้นเมืองไทยมีจุดกำเนิดเดียวกันกับสุกรในประเทศจีนตอนใต้และตะวันตกเฉียงใต้ การศึกษาอีกประการหนึ่งเพื่อเปรียบเทียบลำดับเบสของดีเอ็นเอของยีน cytochrome b ของสุกรไทยภาคใต้ สุกรจีนพันธุ์เชียนเปแบลค และสุกรป่า ผลการศึกษาพบ ความผันแปรของนิวคลีโอไทด์จำนวน 8 ตำแหน่ง สามารถแยกความแตกต่างของแฮโพรไทป์ได้ 5 แบบ โดยพบ แฮโพรไทป์ 1 แบบ (HCS) ในประชากรสุกรไทยภาคใต้ พบแฮโพรไทป์ 3 แบบ (HC1, HC2, และ HCS) ในประชากรสุกรจีนพันธุ์เชียนเปแบลค และแฮโพรไทป์ 2 แบบในประชากรสุกรป่า ซึ่งจะเห็นได้ว่าสุกรจากภาคใต้มีแฮโพรไทป์ 1 แบบ (HCS) ตรงกับสุกรจีนพันธุ์เชียนเปแบลค และการวิเคราะห์ทางไฟโลเจเนติกแสดงว่า สุกรไทยภาคใต้มีความสัมพันธ์ทางพันธุกรรมใกล้ชิดกับสุกรจีนพันธุ์เชียนเปแบลค ซึ่งสอดคล้องกับการอนุมาณที่ว่าสุกรพื้นเมืองไทยอาจมีจุดกำเนิดเดียวกันกับสุกรในประเทศจีนตอนใต้และตะวันตกเฉียงใต้ นอกจากนี้ได้มีการบ่งชี้ตำแหน่งของเอนไซม์ตัดจำเพาะบนแฮโพรไทป์ทั้ง 5 แบบ

การศึกษาเพื่อประเมินความสัมพันธ์ทางพันธุกรรม ด้วยการสร้างไฟโลเจเนติกทรีด้วยวิธี Neibor-Joining โดยการเปรียบเทียบความแตกต่างของลำดับเบสในยีน cytochrome b จำนวน 14 แฮพโพรไทป์ที่เป็นตัวแทนของสุกรไทยภาคใต้ สุกรไทยภาคตะวันออกเฉียงเหนือ สุกรจีนพันธุ์เชียนเปแบลค และสุกรป่า และใช้ 15 แฮพโพรไทป์ที่เป็นตัวแทนของสุกรพันธุ์ต่างประเทศที่มีข้อมูลอยู่ใน Genbank ผลการวิเคราะห์ชี้ว่าสุกรไทยจากภาคใต้และสุกรจีน 5 พันธุ์ (ได้แก่พันธุ์ จินหัว โรงชาง เหมยซาน เสียง เชียนเปแบลค) และสุกรไทยภาคตะวันออกเฉียงเหนือบางส่วนมีความสัมพันธ์ทางพันธุกรรมกันอย่างใกล้ชิด การศึกษาครั้งนี้ชี้แนะว่าสุกรป่าในประเทศไทยอาจจะสามารถจัดอยู่ในกลุ่มพันธุกรรมเดียวกันกับสุกรป่าของเอเชียอาคเนย์อื่น ๆ ได้

สาขาวิชาเทคโนโลยีการผลิตสัตว์ ลายมือชื่อนักศึกษา_____

ปีการศึกษา 2550 ลายมือชื่ออาจารย์ที่ปรึกษา_____

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม_____

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม_____

YANG SHENGLIN : GENETIC DIVERSITY OF THAI INDIGENOUS PIGS, WILD BOARS AND CHINESE QIANBEI BLACK PIGS BASED ON MICROSATELLITE DNA AND SEQUENCE POLYMORPHISM OF MITOCHONDRIA DNA *CYTOCHROME* b GENE. THESIS ADVISOR : ASSOC. PROF. PONGCHAN NA-LAM PANG, Ph.D., 162 PP.

DIVERSITY/INDIGENOUS PIGS/WILD BOARS/CHINESE QIANBEI BLACK PIGS /mtDNA/POLYMORPHISM

The number of Thai indigenous pigs has been rapidly decreasing since exotic breeds were first introduced for breeding improvement in 1960s. Until now, little is known about previous or current genetic variations of indigenous Thai pigs based on molecular level studies. Therefore, the objectives of this study were to find genetic diversity among Southern Thai pigs (ST), Northeastern Thai pigs (NT), wild boars (WB), and Chinese Qianbei Black pigs (CQB), based on microsatellite markers, and to determine the sequences polymorphism of mtDNA cytochrome b gene (*Cyt* b) among these four pig populations. Phylogenetic relationships among these four pig populations based on sequences polymorphism of mtDNA *Cyt* b gene were also studied in this research. A preliminary experiment was conducted to compare different DNA sources from blood and hair root samples for PCR reaction based on microsatellite loci S0225 and S0227 and mtDNA *Cyt* b gene. Results indicted that DNA from all hair root samples could be used as templates for microsatellite PCR, and *Cyt* b gene PCR. Therefore, hair root sample can be used as the DNA source

because sampling method was simple and less harmful to pigs. The major research was to evaluate genetic variations of the twoThai indigenous pig populations using 12 microsatellite primers. NT and ST pig populations exhibited higher average expected heterozygosity (H_E = 0.86 and 0.84) and Polymorphism Information Content (*PIC*) values (0.82 and 0.81) than European pig breeds and some Chinese pig breeds. The four populations studied were in Hardy-Winberg equilibrium (P<0.05). A UPGMA tree based on Nei's D_A standard genetic distances showed that CQB pigs and NT and ST pigs were clustered into the same branches with a 100% bootstrap support value, but WB were clustered into another branch. An inference was made that the Thai native pigs might have the same origin as pigs of south or southwest China. The other study was to examine the sequence polymorphism of ST pigs, CQB pigs and WB pigs and to evaluate the phylogenetic relationships based on *Cyt* b gene fragment; a total of the 5 haplotypes with 8 polymorphic nucleotide sites were detected. Only one haplotype (HCS) was found in ST pigs. Three different haplotypes(HC1, HC2 and HCS) were detected in CQB pigs. There were two haplotypes (HWB1 and HWB2) in WB pigs; furthermore, ST pigs shared the haplotype with the CQB pigs. Additionally, restriction enzyme sites were also identified on 5 haplotypes of *Cyt* b genes. Phylogenetic analysis showed that ST pigs had a close genetic relationship with CQB pigs, which was consistent with the inference that Thai native pigs might have the same origin as pigs of south or southwest China.

Phylogenetic trees were also constructed based on the Neighbor-Joining method using 14 haplotypes representing ST, NT, CQB, and WB pig breeds and 15 haplotypes representing exotic breeds from Genbank. Analytical results indicated that ST pigs and five Chinese domestic pig breeds (including, Jinhua, Rongchang,

Meishan, Xiang pig, Qianbei black) and one northeast Thai pig had closer genetic relationships. The present study suggests that wild boars in Thailand could be put into the same cluster with other Southeast Asian wild boars.

School of Animal Production Technology

Academic year 2007

Student' Signature_____

Advisor' Signature_____

Co-advisor' Signature_____

Co-advisor' Signature_____

ACKNOWLEDGEMENTS

This research could not be completed without the support of many people. With this I would like to express my sincerest appreciation and deepest gratitude to the following persons:

Assoc. Prof. Dr. Pongchan Na-Lampang, advisor, for giving me the chance to be as a Teaching Assistant during my stay at SUT, his kind support, guidance, and editing this thesis until nearly examination of dissertation.

Dr. Surintorn Boonanuntanasarn, co-advisor, for her invaluable guidance on molecular techniques and knowledge as well as her encouragement and suggestions. Special appreciation is given to her friendly connections with the other universities, providing me with the best opportunities to learn molecular techniques at Chulalongkorn University in Thailand and to conduct my partial experiment at Kasetsart University in Thailand.

Professor Uthairat Na-Nakorn, Ph.D, co-advisor, for her kind assistance on experimental environment in Fish Genetic Laboratory, Kasetsart University, including materials and chemical reagents for my research, as well as her invaluable suggestions and comments on my dissertation.

Dr. Pakenit, Dr. Pramote, Dr. Samorn, and other members in School of Animal Production, a very special appreciation is given to them for guidance, their

advice, their kind assistance during the time of my study in Thailand, their warmest hospitality as well.

Miss Nitchanan Chukerd, for her kind assistance in sample collection and laboratory work, particularly in some data providing and analysis. Mr. Phapoom. Mr. Jakpan, and the other Master classmates for their help in sample collection and laboratory work.

Mr. Clifford Sloane, a native speaker of English from United States working at SUT, for having a careful and patient proofreading of my thesis.

Mr. Han Yong, a PhD candidate from Guizhou University of China, my roommate, special thanks to him for his kind care and help in study and life during my stay in Thailand.

Miss Saw, a secretary of School of Animal Production at Suranaree University of Technology, for her help and assistance in some business related to my studies and work at this university.

Miss Srijanya Sukmanomon, Dr. Kednapat Sriphairoj, Miss Anyalak Wachirachaikarn, Miss Thanatip Lamkom, who are pursuing MS or PhD programs in Fish Genetic Laboratory of Kasetsart University, for their guidance and assistances on molecular techniques. Thanks also to Mr. Ha Phuoc Hung, a PhD student from Vietnam studying at Kasetsart University, for his friendly help in daily life and work during my three-month stay at this University.

Mr. Shi Zhong-hui, Deputy Director of Animal Husbandry Bureau of Zunyi, Guizhou Province, China, for his friendly offering with the Qianbei Black pig samples

from Qianbei Black pig Conservation Farm of Zunyi region of Guizhou Province.

Associate Professor Tao Yu-shun, Professor Liu Pei-qiong, Professor Wang Jia-fu, Associate Professor Xia Xian-lin and Associate Professor Luo Wei-xing, as well as my colleagues at Guizhou University, for their support and encouragement throughout the study period.

Finally, my appreciation is devoted to my 77-year old mother, for giving me inspirations, as well as to my wife Weiyan, my daughter Wenqi, my three elder brothers and an elder sister, for their infinite love, patience, sacrifices and understanding during stay for this study.

Yang Shenglin

CONTENTS

Page

CONTENTS (Continued)

Page

CONTENTS (Continued)

Page

CONTENTS (Continued)

Page

LIST OF TABLES

Table **Page**

LIST OF TABLES (Continued)

Table **Page**

LIST OF FIGURES

Figure **Page**

LIST OF FIGURES (Continued)

Figure **Page**

LIST OF FIGURES (Continued)

Page

LIST OF ABBREVIATIONS

ATCG	=	nucleotide containing the base adenine, thymine, cytosine, and guanine, respectively
℃	=	degree Celsius
DNA	=	deoxyribonucleic acid
EDTA	=	ethylene diamine tetraacetic acid
PAGE	=	Polyacrylamide gel electrophoresis
Tris	=	Tris(hydroxyaminometane)
Tris-HCl	=	Tris-hydrochloride
SDS	=	Sodiumdodecyl sulfate
Kb	=	Kilobase
bp	=	base pair
Min	=	minute
mg	=	milligram
ml	=	milliliter
mM	=	millimolar
M	=	molar
$MgCl_2$	=	magnesium chloride
ng	=	nanogram
µL	=	microlitre

LIST OF ABBREVIATIONS (Continued)

Mm	=	micromolar
µg	=	Microgram
h	=	hour
OD	=	optical density
PCR	=	polymerase chain reaction
RNase A	=	ribonuclease A
rpm	=	revolution per minute

CHAPTER I

INTRODUCTION

1.1 Rationale of the study

There are several terms for Thai pigs in different areas. According to Tanaka (1974), there were three types of indigenous Thai pigs, i.e. Hailum, primarily distributed in the southern and the central areas of Thailand; Murad, mostly distributed in the northern, the northeastern and the southern regions in Thailand; Mukuai, mainly in the north and the central areas of Thailand. These three types of Thai native pigs are various in morphological traits; for example, the Hailum pig has a white belly and foot rather than other two types. The Mukuai pig has a larger bodyweight than the others (Tanaka et al., 1974).

Generally, indigenous pig breeds possess valuable traits such as disease resistance, high fertility, good maternal qualities, unique product qualities, and adaptation to harsh conditions and poor quality feed. These are all desirable qualities for achieving sustainable agriculture under low-input conditions. However, one of the problems arising in conservation strategy is that the indigenous pigs consist of several populations localized in the different areas of Thailand. It is not known whether these populations belong to identical breeds.

Previous investigations involved in genetic analysis of the Thailand indigenous pig populations using microsatellite markers (Chaiwatanasin et al., 2002). Tanaka, in his study conducted in1974 on polymorphism of serological protein in

Thai native pig, could not find significant differences among three types of Thai native pigs. The reports of analysis on the genetic relationship between Thai indigenous pigs and Chinese native pigs, and introduced breeds have not been found.

During the last few decades, a variety of different techniques to analyze genetic variation have appeared due to the tremendous developments in the field of molecular genetics. Molecular markers are valuable means to identify animal genetic relationships and levels of polymorphism (Ranguren-Mende, *et al*., 2004). There are many DNA markers that have been applied to study plant and animal genetic diversity but main makers include restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), Microsatellite or simple sequence repeats (SSR), single strand conformation polymorphism (SSCP) etc. These genetic markers may differ with respect to important features: genetic abundance, level of polymorphism detected, locus specificity, reproducibility, technical requirements and financial investment. Therefore, it is not all DNA markers that are suitable for all other range of applications, the choice of the most appropriate genetic marker will depend on the specific application.

Microsatellite, or simple sequence repeats (SSR), is widely used to study the genetic diversity in plants and animals because of the typically neutral, co-dominant (Baumung *et al*., 2004; Vernesi, *et al*., 2003). The high information content of the genetic data produced by microsatellite loci can be sampled from populations. Polymorphism is created by the existence of variants in a given set of samples. Variants can be identified at different interlocked levels of the genetic background: genotype, alleles, haplotypes, and nucleotides. It has been widely used in studies on

animal genetic diversity. In recent years, mitochondrial DNA (mtDNA) has become a useful tool for phylogenetic analysis, and several studies of the relationship between wild boar and domestic pig populations using mtDNA polymorphism have been carried out (Watanobe *et al*., 1999; Okumura *et al*., 2001; Alves *et al*., 2003). The results have revealed that several independent domestications of wild boars have taken place in Europe and in Asia (Giuffra *et al*., 2000; Kijas and Andersson, 2001; Larson *et al*., 2005).

Mitochondrial DNA (mtDNA) has been widely used for phylogenetic studies for several reasons. First, evolution of mammalian mtDNA occurs primarily as single base pair substitutions, with only infrequent major sequence rearrangements (Wolstenholme, 1992). Secondly, the rate of mtDNA evolution appears to be as much as 10 times faster than that of nuclear DNA (Brown *et al*., 1979). Thirdly, mtDNA is maternally inherited, haploid and non-recombining. These features facilitate the use of mtDNA as a tool for determining relationships among individuals within species and among closely related species with recent times of divergence (Avise *et al*., 1979; Brown *et al*., 1979).

In pigs, genetic variability at the cytochrome b gene and the D-loop region has been used as a tool to dissect the genetic relationships between different breeds and populations (Alex *et al*., 2004). Randi *et al*. (1996) used cytochrome b polymorphism for evolutionary analysis of the suiformes and also to determine relationships among some *Sus scrofa* populations. Recently, the complete mtDNA sequence of the pig was published along with its phylogenetic relationships to other animal specie. However, a few studies have been performed on phylogenetic relationships among various pig populations using DNA sequence polymorphism. In

particular, a few studies have conducted on estimates of sequence divergence among different pig breeds from two main domains of the D-loop region and the synonymous and nonsynonymous nucleotide substitutions in the cytochrome B gene.

As to microsatellite markers, a large number of studies have been published on genetic diversity in animals including cattle, sheep, and horse. In pigs, Vernesi *et al*. (2003) studied the genetic diversity using the total number of 105 Italian wild boars and Hungarian wild boars based on 9 microsatellites. Fang *et al*. (2005) investigated for the genetic diversity among Chinese local pigs (32 types), Hainan wild boars, Dongbei wild boars, and exotic species-orkshire using 34 microsatellite markers. The results indicated that Chinese pig breeds have a different origin from European/American breeds and can be utilized in programs that aim to maintain Chinese indigenous pig breeds. In Thailand, Chaiwatanasin *et al*. (2002) investigated the genetic diversity of two Thai native pig populations (the North and the Northeast Thai pigs) using 15 microsatellites. The results indicated that genetic diversity of the northeast native pig was higher than that of the north native pigs. In fact, there are several types of native pigs existing in different areas in Thailand. In the past, some Chinese Meishan pigs, Hailand pigs, Jinhua pigs were introduced into Thailand. Probably, the number of indigenous pig decreased and produced some crossbreeds. Few research reports with respect to genetic characteristics and genetic diversity based on these indigenous breeds have been published. These studies are necessary because they are related to the realm of animal genetic resource conservation in Thailand.

1.2 The overall objectives

The objectives of this study are,

1.2.1 To Study on genetic diversity among Thai pigs, wild boars and Chinese pigs based on microsatellites.

1.2.2 To determine the phylogenetic relationships among Thai pigs, wild boars and Chinese pigs using microsatellite data.

1.2.3 To Study on genetic diversity among South Thai pigs, wild boars and Chinese pigs using sequnce polymorphism of *Cyt* b gene.

1.2.4 To analyse phylogenetic relationships among Thai pig populations and exotic pig breeds using sequnce polymorphism of *Cyt b* gene.

1.3 References

Alex, C., A. Marcel, Jose-Luıs, N. Ana, F. Juan, C. Maria, M. R. Lucıa, K. James, M.H. K. Leif, A. and Armand, S. (2004). Estimating the frequency of Asian cytochrome B haplotypes in standard European and local Spanish pig breeds. **Genet. Sel. Evol.** 36: 97–104.

Alves, E., Ovilo, C. Rodrıguez, M.C., and Silio, L. (2003). Mitochondrial DNA sequence variation and phylogenetic relationships among Iberian pigs and other domestic and wild pig populations. **Anim. Genet.** 34: 319–24.

Avise, J. C., Lansman, R. A., and Shade, R. O. (1979). The use of restriction endonucleases to measure mitochondrial DNA sequence relatedness in natural populations. I. Population structure and evolution in the genus Peromyscus. **Genetics.** 92: 279-95.

Baumung, B. R., Simianer, H., and Hoffmann, I. (2004). Genetic diversity studies in farm animals – a survey. **J. Anim. Breed. Genet.** 121: 361-373.

Brown, W.M., George, M.J., and Wilson, A.C. (1979). Rapid evolution of mitochondrial DNA. **Proceedings of the National Academy of Sciences of USA**. 76: 1967-71.

Chaiwatanasin, W., Somchai, C., Srisuwan, C., Neramit, S., and Sompoch, T. (2002). Genetic Diversity of Native Pig in Thailand Using Microsatellite Analysis. **Kasetsart J. (Nat. Sci.)** 36 : 133 -137.

Fang, M., Jiang, Hu., Braunschweig, X., Hu, T. Du, M., Feng, L., Zhang, Z., Wu, J., and Li. N. (2005). The phylogeny of Chinese indigenous pig breeds inferred from microsatellite markers. **Anim. Genet.** 36: 7-13.

FAO. (1998). Intergovernmental Technical Group Working on Animal Genetic Resources for Food and Agriculture. **1st session, Rome.** pp: 1-12.

Giuffra, E., Kijas, J. M. H., Armager, V., Carlborg, O., Jeon, J.T., and Andersson, L. (2000). The origin of the domestic pig: independent domestication and subsequent introgression. **Genetics.** 154: 1785-91.

Kijas, J.M.H., Wales, R., and Tornsten, A. (1998). Melanocortin receptor 1 (MC1R) mutations and coat color in the pig. **Genetics.** 150: 1177–85.

Larson, G., Dobney, K., and Albarella, U. (2005). Worldwide phylogeography of wild boar reveals multiple centres of pig domestication. **Science.** 307: 1618–21.

Okumura, N., Kurosawa, Y., and Kobayashi, E. (2001). Genetic relationship amongst the major non-coding regions of mitochondrial DNAs in wild boars and several breeds of domesticated pigs. **Animal Genetics.** 32: 139–47.

Randi, E., Lucchini, V., and Diong, C.H. (1996). Evolutionary genetics of the suiformes as reconstructed using mtDNA sequencing. **Journal of Mammalian Evolution**. 3: 163-94.

Tanaka, Kazue. (1974). Morphological and serological studies on the native pigs in Thailand. **Report of the Society for Research on Native Livestock.** No. 6. pp. 181-183.

Vernesi, C., Crestanello, B. Pecchioli, E. Tartari, D. Caramelli, D. Hauffe, H., and Bertorelie, G. (2003). The genetic impact of demographic decline and reintroduction in the wild boar (*Sus scrofa*): Amicrosatellite analysis. **Molecular Ecology.** 12: 585-595.

Watanobe, T., Okumura, N. Ishiguro, N., Nakano, M., Matsui, A., Sahara, M., and Komatsu, M. (1999). Genetic relationship and distribution of the Japanese wild boar (Sus scrofa leucomystax) and Ryukyu wild boar (Sus scrofa riukiuanus) analyzed by mitochondrial DNA. **Molecular Ecology.** 8: 1509-12.

Wolstenholme, D.R. (1992). Animal mitochondrial DNA: structure and evolution. **International Review of Cytology**. 141: 173-215.

CHAPTER II

REVIEW OF THE LITERATURE

2.1 Background of pig industry

Pork has become the second most important meat in Thai consumption, with average consumption in the late 1990s of about 4.7 kg per person per year (FAO, 2002). Pig production started in 1960 when the first group of exotic pig breeds was imported by the Department of Livestock Development from the United Kingdom. These were Large Whites, Tamworth and Berkshire breeds. Later, Landrace and Duroc Jersey breed pigs were imported from the United States. Up until these exotic breeds were introduced, farmers relied on the relatively slow growing native pigs that had the desirable quality of not needing much in the way of traded inputs. The imported pigs were used for breeding improvement and were cross bred with the native pigs (Kanto 1991). Throughout the 1960s and 1970, crossbred pigs were raised by backyard producers for consumption by the farm family and also as a source of income.

The number of pig population is mainly distributed in the central region of Thailand, which has about 50 percent of Thailand's pigs. The Southern region has the smallest number of pigs, possibly reflecting the higher cost of pig fattening because of a shortage of feed in this region. An additional explanation could be that the southern part of Thailand has a relatively high Muslim population for whom consuming pork is prohibited (APHCA, 2002).

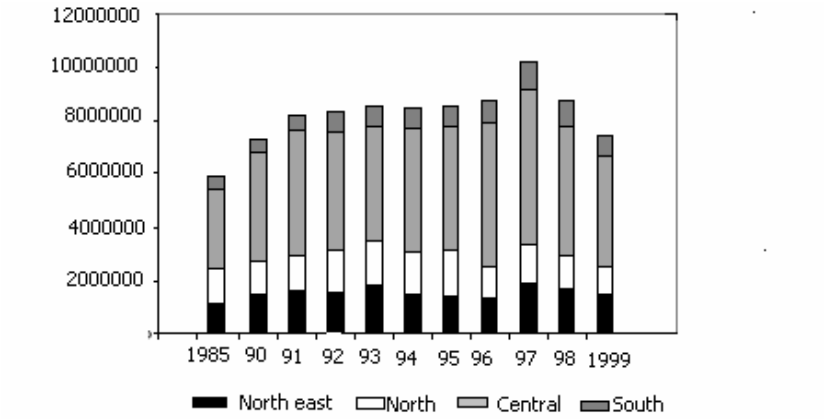

Figure 2.1 Distribution of pigs by regions (Source: APHCA of FAO, 2002.)

Table 2.1 Numbers of holdings rearing swine, Thailand 1993

Swine per holding	Holdings		Swine	
	Number	Percent	Number	Percent
1 – 2	286866	48.57	423119	6.84
3 – 4	86483	14.64	289120	4.67
5 – 9	98163	16.62	630927	10.20
10 - 19	71585	12.12	898307	14.52
20 - 49	34578	5.85	932947	15.08

Source: (APHCA, 2002)

2.2 Basic situation for Thai native pigs

Although the number of crossbred pigs has increased since introduced breeds were used to improve the productive ability of native pigs, there have been a number of native pig populations distributed in different regions of Thailand, mainly reared in north east, and north of Thailand (Chaiwatanasin *et al.*, 2002).With the development of comprehensive pig farms, the native pigs have gradually become rare. Some outstanding traits like good quality of pork will be lost if we do not take measures to protect these animals.

According to Takana (1981), there have been three types of indigenous Thai pigs. Hailum, primarily distributed in the south and the central areas of Thailand; Murad, mostly distributed in the north, the northeast and the south in Thailand; and Mukuai, mainly found in the north and the central areas of Thailand. Their appearance characteristics can be described in Figure 2.2, and their body size can be summarized in Table 2.2.

Table 2.2 Basic body size of 2.5-3 years old Thai native sows

Index	**Hainum(cm)**	**Murad(cm)**	**Mukuai(cm)**
Body length	101.40	86.6	127.4
Body Height	58.1	52.7	70.30
Circumference	97.6	85.3	130.1

Applied from: Protection and utilization manual for animal development in Thailand. 1999-2003

Similarly, some Chinese pig breeds were introduced to improve the genetic gain, such as Meishan, Jinhu, and Hailan etc. It is also said that Hailum pig came from Hainan island of China, but there is no evidence to prove this. Accordingly, native pig populations likely contain other Chinese pig breeds that came from other provinces of China. Further studies are necessary to confirm these conjectures.

2.3 Genetic diversity and genetic variations

2.3.1 Genetic diversity

Information concerned with the genetic diversity of a species comprises variation of genes (hereditary unit) at individual's level within a population or variation between geographical populations. The level of genetic diversity is usually different from one individual to another within a population, and consequently different populations of the same species can differ from one another (Halliburton, 2004). The differences are the result of evolutionary process that reflects adaptation to different conditions of life, locale, and history (Ayala, 1982). Therefore, genetic diversity of a species is an invaluable resource that enables sustainability of the species, and moreover, it is a basic need for successful genetic improvement program.

2.3.2 Measures of genetic diversity

To understand genetic diversity within a breed, one must be able to describe and quantify genetic variation in a population and the pattern of genetic variation among populations. Genetic variation within a population is revealed by average number of alleles per locus, average heterozygosity per individual and proportion of polymorphic loci (Hedrick, 1999).

The mean number of alleles (n_a) per locus is the measure of allelic richness, which is equal to the sum of the count of the number of alleles at all loci divided by number of loci examined. Effective number of alleles (n_e) is the measure of allelic evenness. which is estimated by the formula $1/\Sigma\ p_1^2$, where p_1 is the i^{th} allele frequency (Hedrick, 2000).

Heterozygosity is defined as relative frequency of the heterozygous individuals per one locus. It is calculated as a proportion of actual number of heterozygotes to total number of samples under study. Nei and Kumar (2000) proposed level of heterozygosity as level of gene diversity (h) which was calculated as:

$$H = 1 - \sum_{i=1}^{q} x_i^2$$

Where x_i is a frequency of the i^{th} allele in a population and q is the number of alleles. Since more than one locus is studied, average gene diversity is the average of this quantity over all loci.

Low heterozygosity is normally a consequence of drastically reduction of effective population size (bottleneck). This may finally result in inbreeding, thus reducing individual fitness in a population and increases the chance of extinction of the population. However, some populations may well survive with low heterozygosity such as a population of northern elephant seal (H0 = 0.00019) but this population may not survive if change in environment occurs (Hoelzel, 1999).

A proportion of polymorphic loci are calculated straightforwardly. If two

or more alleles at one locus occur with appreciable frequency, then this locus is considered as polymorphic. In a study with sample less than 100, a locus is considered polymorphic when bearing more than one allele with a maximum frequency not exceeding 0.95.

Polymorphism Information Content (*PIC*) value is a measure of polymorphism introduced by Botstein *et al*. (1980), which gives an indicator of how many alleles a certain marker has and how much these alleles divide evenly. It is calculated by the formula:

$$PIC=1-\sum_{i=1}^{m} pi^2 -\sum_{i=1}^{m-1}\sum_{j=i+1}^{m} 2pi^2 pj^2$$

Where, *pi, pj* represent i^{th} and j^{th} allele frequency at locus i and j, m denotes the number of alleles. If *PIC*>0.5, the loci will be regarded as high polymorphic. If $0.5 > \mathrm{PIC} > 0.25$, it will be medium polymorphic, when $PIC < 0.25$, it will be regarded as low polymorphic.

A number of measures of genetic distance have been suggested over the past several decades. These measures help to consolidate the data into manageable proportions and aid one in visualizing general relationships among the group of populations (Hedrich, 1999). Nei (1972) cited by Hedrick (1999) developed a genetic distance measure called **Standard Genetic Distance** on the following equations. The first step is to calculate genetic identity for a single locus with *n* allele.

$$I=\frac{J_{xy}}{\left(J_x J_y\right)^{\frac{1}{2}}}$$

Where $J_{xy} = \sum_{i=1}^{n} p_{ix} p_{iy}$; $J_x = \sum_{i=1}^{n} p_{ix}^2$ and $J_y = \sum_{i=1}^{n} p_{iy}^2$ and $p_{i.x}$ and $p_{i.y}$ are the frequencies of the i^{th} allele in population x and y respectively. The genetic distance between two populations is then defined as:

$$D_N = - ln (I)$$

For multiple loci, J_{xy}, J_x and J_y values are calculated by summing over alleles at all loci included in the study. The average value per locus is then calculated by dividing these sums by the number of loci. These average values, $\hat{J}_{xy}$, $\hat{J}_x$, and $\hat{J}_y$, are then used to calculate the genetic identity $\bar{I}$, and the distance becomes:

$$\check{D}_N = - ln (\bar{I}_N)$$

Based on molecular-taxonomic survey by using protein electrophoresis analysis in fish, Shaklee *et al*. (1982) found that average Nei's standard genetic distance between conspecific populations was 0.05 (ranged between 0.002–0.065), between congeneric species was 0.30 (0.025–0.609) and between confamilial genera was 0.90 (0.580–1.21). These survey findings agree with Ayala *et al*. (1974) that the degree of genetic distance depends on the levels of evolutionary divergence between related populations or taxa.

Nucleotide diversity (π) is a concept in molecular genetics which is used to measure the degree of polymorphism within a population. It was first introduced by Nei and Li (1979). It is defined as the average number of nucleotide differences per

site between any two DNA sequences chosen randomly from the sample population, and is denoted by π. It is given by the formula:

$$\pi = \sum_{ij} X_i X_j \pi_{ij}$$

In which π_{ij} is the proportion of different nucleotides between the *ith* and *jth* types of DNA sequences, and x_i and x_j are the respective frequencies of these sequences.

The summation is taken over all distinct pairs *i*, *j*, without repetition. That is:

$$\pi = \sum_{ij} x_i x_j \pi_{ij} = \sum_{i=1}^{n} \sum_{j=1}^{i} x_i x_j \pi_{ij}$$

Where *n* is the number of sequences in the sample.

The method of **Phylogenetic Inference** currently used in molecular phylogenetics can be classified into three major groups: distance methods, likelihood methods, and parsimony methods. Recently, Hendy and colleagues (Hendy and Charleston 1993; Hendy and Penny. 1989; Hendy et al., 1994.) proposed the use of the Hadamard conjugation for phylogenetic reconstruction (closest tree method). However, its practical utility is yet to be examined.

In **Distance Methods,** an evolutionary distance is computed for all pairs of sequences, and a phylogenetic tree is constructed from pairwise distances by using the least squares, minimum evolution, or some other criteria. The evolutionary distance

used for this purpose is usually an estimate of the number of nucleotides or amino acid substitutions per site, but other distance measures may also be used. There are a large number of distance methods for constructing phylogenetic trees (Felsenstein, 1988; and Nei, 1987), but those commonly used are based on the principles of least squares and minimum evolution.

In **Maximum Parsimony (MP) Methods**, a given set of nucleotide (or amino acid) sequences are considered, and the nucleotides (or amino acids) of ancestral sequences for a hypothetical topology are inferred under the assumption that mutational changes occur in all directions among the four different nucleotides (or 20 amino acids). The smallest number of nucleotide substitutions that explain the entire evolutionary process for the given topology is then computed. This computation is done for all other topologies, and the topology that requires the smallest number of substitutions is chosen to be the best tree (Fitch, 1971 and Hartigan 1973).

Statistical tests of phylogenetic trees can be divided into two categories: a test of reliability of a tree obtained and a test of topological differences between two or more different trees obtainable from the same data set. One of the most commonly used tests of the reliability of an inferred tree is Felsenstein's **Bootstrap Test** (Felsenstein, 1985). In this test, the reliability of an inferred tree is examined by using Efron's bootstrap resampling technique (Efron, 1982). A set of nucleotide sites is randomly sampled with replacement from the original set, and this random set is used for constructing a new phylogenetic tree. This process is repeated many times, and the proportion of replications in which a given sequence cluster appears is computed. If this proportion (*PB*) is high (say, $PB > 0{:}95$) for a sequence cluster, this cluster is

considered to be statistically significant.

2.4 Molecular markers for evaluating genetic diversity

Molecular markers are valuable means to identify animal genetic relationships and levels of polymorphism (Ranguren-Mende, *et al.*, 2004). There are many DNA markers that have been applied to study plant and animal genetic diversity but mainly focus on several ones such as FFLP, RAPD, AFLP, Microsatellite, and SSCP etc. among them, Microsatellite is widely used to study the genetic diversity in plants and animals because of its high information content of the genetic data produced by microsatellite loci (Baumung *et al*., 2004). In recent years, mitochondrial DNA (mtDNA) has become a useful tool for phylogenetic analysis due to the quicker rate of mtDNA evolution. Here, the properties of microsatellite and mtDNA markers will be mainly described.

2.4.1 Microsatellite Marker

2.4.1.1 Properties of microsatellites

Microsatellites are short segments of DNA that have a repeated sequence such as CACACACA, and they tend to occur in non-coding DNA (Weber, 1990). In some microsatellites, the repeated unit (e.g. CA) may occur 4 times; in others it may be 7, or 2, or 30. The most common way to detect microsatellites is to design PCR primers that are unique to one locus in the genome and that base pair on either side of the repeated portion (Figure 2.2). Therefore, a single pair of PCR primers will work

for every individual in the species and produce different sized products for each of the different length microsatellites.

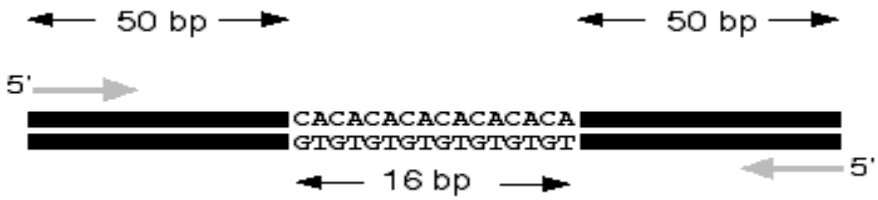

Figure 2.2 Detecting microsatellites from genomic DNA. Two PCR primers (forward and reverse gray arrows) are designed to flank the microsatellite region. If there were zero repeats, the PCR product would be 100 bp in length.

Microsatellites are widely dispersed throughout eukaryotic genomes and are often highly polymorphic due to variation in the number of repeated units. The high information content of the genetic data produced by microsatellite loci can be sampled from different populations. In addition, a potentially valuable characteristic of microsatellite is that primers developed on one species can be used in related populations. This is particularly important for studies in ecology and in conservation of endangered species.

To identify animal genetic diversity using microsatellite makers is more precise and effective than that using traditional methods such as cytogenetic and biochemical methods (Baumung *et al*., 2004). The individual genotypes can be obtained with the aid of the property of polymorphism and codominance of microsatellite DNA. The allele frequencies, mean heterozygosity and effective

number of alleles can be calculated. The genetic distance and dendrogram can also be analyzed by means of principles of quantitative genetics and molecular genetics, so as to analyze the variance degrees of populations and genetic relationships. Compared with the dendrogram based on the polymorphic protein markers, the dendrogram constructed by microsatellite markers is more consistent with the history and distribution of animal population (Baumung *et al*., 2004).

2.4.1.2. Applications of microsatellites

Microsatellites have been proposed as the best markers for evaluating the genetic diversities of domestic animals because of their abundant, even distribution in the genome, high polymorphism and ease of genotyping. The International Society of Animal Genetics (ISAG) and FAO have recommended a set of 27 microsatellite loci (http://www.toulouse.inra.fr/lgc/pig/panel/html) for evaluating the genetic diversities of pigs as part of the global strategy for the management of farm animal genetic resources (Hammond and Leitch, 1998). If all researchers adopt the same markers, results will be comparable.

During the past decades a large number of genetic diversity studies in domestic livestock based on microsatellite loci were carried out all over the world (Baumung *et al.*, 2004). Microsatellite can successfully explain the relationships between both individuals and populations. More particularly, they are commonly used to assess diversity within breeds, inbreeding levels, breed differentiation, introgression or breed admixture. Most microsatellite population genetic studies are limited to small numbers of breeds, often from a single country (Arranz *et al*., 1998; Li *et al.*, 2002; Baumung *et al*., 2004), but several studies have examined diversity

and distribution of livestock at the regional level or even at the scale of nearly an entire continent (Hanotte *et al*., 2002). The majority of papers were related to cattle. One of the total 19 adopted 50 breeds from 23 countries (Hanotte *et al*., 2002). The smallest number of breeds was only 3 from one country (Dorji *et al*., 2003). The smallest sample size was 10 while the largest sample size was 83 (MacHugh *et al.*, 1997). Up to year 2006, more than 10 (not including Chinese publications) of the studies on genetic diversity in pigs based on microsatellite have been found (Table 1). In these smallest number of breeds was 2, and the largest one was 65 referred to 16 countries (SanCristobal *et al.*, 2002), the smallest sample size was 10 while the largest one was 67. Many studies adopted the microsatellite markers as recommended by FAO / ISIG. Only 1 paper used AFLP to analyse genetic diversity.

2.4.2 Mitochondrial DNA

2.4.2.1 Structure of mitochondria DNA

Mitochondria are a small energy-producing organelle found in the cells. It has its own DNA molecules, entirely separate from nuclear DNA. Most cells contain between 500 and 1000 copies of the mtDNA molecule, which makes it much easier to find and extract than nuclear DNA. In humans the mtDNA genome consists of about 16 kb (far shorter than human nuclear DNA), and has been completely sequenced (Anderson *et al*., 1981). Pig mtDNA is a 16 kb circular molecule including 13 protein-coding genes, 22 tRNA and genes responsible for 12S and 16S rRNA (Kim *et al.*, 2002).

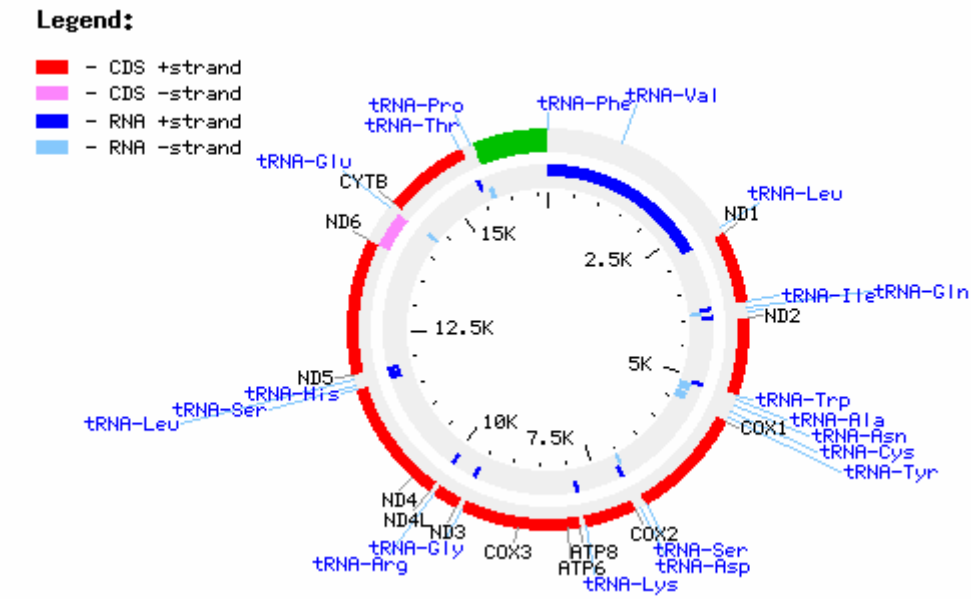

Figure 2.3 Vertebrate mitochondrial DNA (mtDNA) the mtDNA genome is a small, circular molecule, about 16 ~ 18,000 bp in circumference in most vertebrate species. The genome comprises 13 protein-coding regions, two rRNA genes, a replication control region, and 22 tRNA genes. The order of these is broadly conserved across vertebrates. There are no introns: splicing out of tRNAs produces mRNA templates. The mtDNA genome is self-replicating with the aid of nucDNA-encoded polymerases. It contributes to cell respiratory systems in the Cytochrome Oxidase, ATP synthase, and NADH systems. The vertebrate mtDNA genetic code differs from the "Universal" code is several respects.

Source: www.mun.ca/biology/scarr/mtDNA_genome.html

Mitochondrial DNA (Figure 2.3) is the only genetic material that exists

outside of animal nucleolus (Wolstenholme *et al*., 1992). It is a circular and super-coiled molecule (Brown et al., 1979). It has been widely used to study molecular evolution, biological classification and population genetic structure due to its small molecular weight, the quick evolution rate, almost exclusively maternally inheritance and absence of genetic recombination. It also has been used for those studies to diagnose human disease and to analyze the economical characters of domestic animals (Wallace, 1993).

2.4.2.2 Genetic characteristics of mitochondria DNA

1) Mitochondria DNA has a feature of half independence

Mitochondria has its own genetic material, thus it is one kind of half-independent duplicates, indicating that mtDNA is independently able to duplicate, to transcript and to translate. However, the functions of mtDNA are affected by nuclear DNA as it encodes macromolecular compounds and proteins that can maintain the structure and functions of mtDNA (Brown *et al.*, 1979).

2) The genetic codes of mtDNA genomes are different from the common genetic codes in nuclear genomes

Unlike genomic DNA, UGA is the code of tryptophan rather than stop codon. Methionines (Met) in polypeptide are encoded by both AUG and AUA (Brown et al., 1983), while initial methionines are encoded by four codons -AUG, AUA, AUU and AUC. AGA and AGG are stop codons rather than codons of arginine (Arg). There are four stop codons (UAA, UAG, AGA and AGG) in mitochondria DNA).

3) mtDNA is maternally inherited

In most animal species, mitochondria appear to be primarily inherited

through the maternal lineage, though some recent evidence suggests that in rare instances mitochondria may also be inherited in a paternal route. Typically, a sperm carries mitochondria in its tail as an energy source for its long journey to the egg. When the sperm attaches to the egg during fertilization, the tail falls off. Consequently, the only mitochondria the new organism usually gets are from the egg its mother provided (Brown. *et al*., 1983) Therefore, unlike nuclear DNA, mitochondrial DNA doesn't get shuffled every generation, so it is presumed to change at a slower rate, which is useful for the study of human evolution.

4) The high mutation rate of control region in mtDNA

D - loop is control region of mtDNA, with a highly content of base A and T, for this noncoded region, it approximately composes 6 % mtDNA genome (Brown. et al., 1994). In the pig, D-loop is located between $tRNA^{pro}$ and $tRNA^{phe}$ (Figure 2). It contains 5-29 of Tandem Repeated Sequence (TRS) and its basic base order is CGTGCGTACA., which located between Conserved Sequence Block 1 (CSB – 1) and Conserved Sequence Block 2 (CSB - 2). With respect to evolution, substitution rate of D - loop base is 5 ~ 10 times higher than other regions (MacKay *et al.*, 1986). D - Loop is the highest mutation region in mtDNA molecular.

2.4.2.3 Related studies on polymorphisms of mtDNA in pigs

Animal mitochondrial DNA (mtDNA) is highly polymorphic, almost exclusively maternally inherited and without genetic recombination. The clonal transmission of mtDNA haplotypes allows the discrimination of maternal lineages within species and the analysis of sequences of their most variable regions can be used to investigate the genetic origin of animal populations and breeds and thus the

domestication process of livestock species (Bradley *et al.*, 1996; Luikart *et al*., 2001).

Most of the previous studies were to determine the phylogenetic relationships among varieties of pig populations by using direct sequencing of the main non-coding mtDNA region (D-loop) and cytochrome b gene (Cyt b). Randi *et al*. (1996) used cytochrome b polymorphism for evolutionary analysis of the suiformes and also to determine relationships among some *Sus scrofa* populations. Alves *et al*. (2003) used nucleotide sequences of cytochrome b gene (1140 bp) and control region (707 bp) to determine the phylogenetic relationships among 51 pig samples representing ancient and current varieties of Iberian pigs. A neighbour-joining tree constructed from pairwise distances provides evidence of the European origin of both Iberian pigs and Spanish wild boars. Four estimates of sequence divergence between European and Asian clades were calculated from the two main domains of the D-loop region and the synonymous and nonsynonymous nucleotide substitutions in the cytochrome b gene.

Alex *et al*. (2004) analysed four SNP at the cytochrome b gene to infer the Asian (A1 and A2 haplotypes) or European (E1 and E2 haplotypes) origins of several European standard and local pig breeds, and found a mixture of Asian and European haplotypes in the Canarian Black pig , German Pi´etrain, Belgian Pi´etrain, Large White and Landrace breeds. Recently, Giuffra *et al.* (2000) provided comprehensive molecular analyses regarding the genetic relationship between domestic pigs and wild boars; this analysis included the mtDNA *Cyt* b gene, the major non-coding region of mtDNA, and three nuclear genes (melanocortin receptor 1 [MC1R], tyrosinase [TYR], and the glucose phosphate isomerase pseudogene [GPIP]. These authors presented clear evidence of the independent domestication events of European and Asian subspecies of wild boar. Their conclusion regarding these domestication events is

essentially the same as that of Watanobe *et al.* (1999), who relied on an analysis of the entire major non-coding region of mtDNA. However, the phylogenetic analyses of these previous studies do not include outgroup comparison, which is necessary to assess inner group relationships among individuals from wild boars and domestic pigs.

For Chinese indigenous pig breeds, studies of porcine diversity have often considered only one or a small number of Chinese indigenous breeds (Giuffra *et al*., 2000). Studies were mainly focused on a relatively small region of the mtDNA control region (Kim *et al.*, 2002; Okumura *et al.*, 2001). Variable substitution rates both between mtDNA components (Zardoya and Meyer, 1996) and between lineages mean that an increasing number of studies are based on the entire mtDNA genome (Kijas, 2001).

Reports have not been found to analyze genetic relationship of indigenous Thai pig populations by using mtDNA sequence polymorphism of control region and cytochrome b gene.

2.5 Studies on genetic diversity in pig in Asia

In the past decades, some reports related to genetic diversity in Asian pigs have been noted. These studies primarily conducted in China, Japan, Thailand, South Korea, India, Vietnam, Laos and so forth.

In China, the native pigs almost exist in every province, and each province has their pig strains. Zhang *et al.* (2003) surveyed the genetic diversity of 56 indigenous breeds in China and 3 introduced pig breeds (Duroc, Landrace, and Large White) using 27 microsatellites recommended by FAO and ISIG. By means of allele

frequencies, heterozygosity, effective number of alleles, estimator of gene differentiation, polymorphism information content, genetic distance and dendrogram analyses, the variability of native pig breeds were estimated. Fifty-six Chinese native pig breeds were clustered into 12 groups based on the dendrogram. In 2005, A genetic study of 32 local Chinese, three foreign pig breeds [Duroc (DU)], Landrace and Yorkshire], and two types of wild boar (Hainan and Dongbei wild boar) based on 34 microsatellite loci was carried out to clarify the phylogeny of Chinese indigenous pig breeds (Fang *et al.*, 2005). The allele frequencies, effective numbers of alleles, and the average heterozygosity within populations were calculated. The results only partly agree with the traditional types of classification and also provide a new relationship among Chinese native pig breeds. The data also confirmed that Chinese pig breeds have a different origin from European/American breeds and can be utilized in programmes that aim to maintain Chinese indigenous pig breeds. There are some miniature pig breeds such as Wuzhishan pig, and Xiang pig, which possess specific characteristics. They are considered useful for medical and veterinary research due to their small size. Normally, a mature adult weights less than 25 kg. More recently, Wang *et al*. (2006) estimated genetic polymorphism in 4 inbreeds using 30 Microsatellite genes, the results indicated a relatively high degree of heterozygosity, perhaps because these strains were inbred for 3 generations.

In India, three main types of domesticated pigs have been described: Desi, Gahuri and Ankamali, inhabiting northern India, north-eastern India and Kerala province located in southern India respectively (Bhat *et al.* 1981). Although the growth rate and feed conversion ratio of native Indian pigs including Ankamali pigs is less than those of the exotic or crossbred pigs (Kumar *et al*. 1990; Gaur *et al*. 1997),

they have unique features such as disease resistance, heat tolerance and ability to produce meat with less fat when compared with exotic breeds (Chhabra *et al*. 1999). Based on the above information, Behl R. *et al*. (2006) determined genetic characteristics of Ankamali pigs in Kerala, using 23 FAO recommended microsatellite markers and compared these with other native Indian pig types and Large White pigs. Relevant genetic variations have been obtained.

Table 2.3 Recent publications in studies of genetic diversity based on microsatellite DNA analysis in pig

References	Breed	Sample size (min-max)	Marker used	Primers number used
Behl et al. (2006)	1 Indian pig breed; 1 Large White pig breed.	26-45	Microsatellite	23 Recommended by FAO
Chaiwatanasin et al. (2002)	2 types of Thai native pigs.	22-27	Microsatellite	15 selected by authors
Fan et al. (2002)	7 types of Chinese native pigs	16-65	Microsatellite	27 Recommended by FAO
Fang et al. (2005)	32 types of Chinese pigs; 2 types of Chinese wild boars; 3 foreign pig breeds.	8-30	Microsatellite	34 containing 17 primers recommended by FAO /ISAG
Geldermann et al. (2004)	5 Vietnamese native pig breeds; 3 European pig breeds; 1European wild boars.	17-32	Microsatellite	10 Recommended by FAO
Kim et al. (2002)	1 Korean native pig; 1 Chinese pig; 1 Japanese pig; 3 exotic breeds.	8-10	AFLP	Three ***EcoR I /Taq I*** primer combinations
Kim et al. (2005)	2 Korean pig breeds; 3 Chinese pig breeds; 4 European pig breeds.	12-32	Microsatellite	16 selected by authors
Lemus-Flore et al. (2001)	4 types of Mexican hairless pigs; 4 Commercial pig breeds	10-44	Microsatellite	10 recommended by FAO/ISAG
Li et al. (2000)	4 Chinese pig breeds; 1 Australia pig	11-23	Microsatelite	27 recommended by FAO

Also, there are some native pig breeds in Korea; two kinds of molecular makers have been reported to be used to study Korean native pigs. Kyung *et al*. (2002) assess the genetic diversity and genetic relationships among the six commercial pig breeds including Korean native pig. They performed an amplified fragment length polymorphism (AFLP) analysis. Applying the three *EcoR I/Tag I* primer recombination to 54 individual pig samples out of six breeds. A total of 186 AFLP bands were generated. 67 (33%) were identified as polymorphic bands. From all the calculations of genetic diversity, the lowest genetic diversity was exhibited in the Korean native pig, and the highest in the Chinese Yanbian native pig. In 2005, in order to understand molecular genetic characteristics of Korean pigs, Kim et al. studied the genetic relationships of nine pig breeds including two Korean pigs (Korean native pig and Korean wild pig), three Chinese pigs (Min pig, Xiang pig, and Wuzhishan pig), and four European breeds (Berkshire, Duroc, Landrace, and Yorkshire) based on 16-microsatellite loci analysis. The mean heterozygosity within breeds ranged from 0.494 to 0.703. Relationship trees based on the Nei's DA genetic distance and scatter diagram from principal component analysis consistently displayed pronounced genetic differentiation among the Korean wild pig, Xiang pig, and Wuzhishan pig. These results indicated that the Korean native pig has been experiencing progressive interbreeding with Western pig breeds after originating from a North China pig breed with a black coat color.

In Thailand, the native pigs main distribute in northeast, in the past twenty years, a large number of native pigs have been disappeared because of the increase of introduced species. The conservation of genetic diversity has become more and more important. Accordingly, previous investigation involved in genetic analysis of the

Thailand indigenous pig populations using microsatellite markers has been reported (Chaiwatanasin *et al*., 2002). However, samples for this research were taken only from northeast and north of Thailand, which could not represent whole native pig population in Thailand. The study on polymorphism of serological protein in Thai native pig was conducted by Tanaka (1974), could not show significant differences among three types of Thai native pigs. The reports of analysis on the genetic relationship among Thailand indigenous pigs and Chinese native pigs, and introduced breeds have not been found.

In Japan, a report with respect to the origin of the Ryukyuan native pigs has been found (Tomowo, 2000). The mitochondrial cytochrome b gene (1140bp) of twenty four individuals of Ryukyuan native domestic pigs(*Sus scrofa*) in Okinawa and Amami Islands, southwestern Japan, two individuals of Thaiwanese short ear native pigs, and two individuals of the Kinhua pig in central China were determined. Two different sequence types, namely the Asian pig type and European pig type, were found among the individuals raising in Okinawa and Amami Islands. The cytochrome b gene sequence of the Asian pig type was completely identical with that of Chinese breeds, the Meishan pig and the Kinhua pig. These results indicted that the Ryukyuan native pigs were introduced from China in ancient time.

The native pigs in Laos, in most cases, were pigs of the short ear type but some pigs with large pendulant ears were found in this particular pig population (Yaetsu *et al*., 2000). Tomowo *et al*. (2000) determined the mitochondrial cytochrome b gene sequences (1140 bp) of four individuals of the wild boar and two individuals of the native domestic pig (*Sus scrofa*) in Laos and Vietnam. The phylogenetic analysis revealed that *Sus srofa* in Asia consisted of several evolutionary lineages.

The wild boars in Laos were subdivided into two subspecific groups. An individual from Xiengkhuang Province, approximately 160 km NE of Vientiane was shown to be more closely to the Taiwanese wild boar than to other individuals of the Laotian and Vietnamese wild boars. The cytochrome b gene sequence of native domestic pigs in Laos and Vietnam was completely identical with that of the Meishan pig, a Chinese breed, suggesting that both pigs had a late common ancestor.

So far, in Vietnam, there are about ten Vietnamese indigenous breeds listed in the FAO inventory. Prof. Dr. Geldermann (2004) analysed the genetic diversity using 10 microsatellites among five Vietnamese indigenous pig breeds and two exotic breeds in Vietnam, three European commercial breeds and European Wild Boar were included. Some genetic variations have been acquired from this research.

2.6 References

Alex, C., Marcel, A., Jose-Luıs, N., Ana, F., Juan, C., Maria, M. R., Lucıa, K., James, M.H. K., Leif, A., and Armand, S. (2004). Estimating the frequency of Asian cytochrome B haplotypes in standard European and local Spanish pig breeds. **Genet. Sel. Evol.** 36: 97–104.

Alves, E., Ovilo, C., Rodrıguez, M.C., and Silio, L. (2003). Mitochondrial DNA sequence variation and phylogenetic relationships among Iberian pigs and other domestic and wild pig populations. **Anim. Genet.** 34: 319–24.

Anderson, S., Bankier, A.T., Barrel, B.G., Bruijn, M. H. L., Coulson, A. R., and Drouine, J. (1981). Sequence and organization of the human mitochondrial genome. **Nature.** 290:457-74.

Animal Production and Health Commission for Asia and the Pacific (APHCA) of FAO, 2002. **Livestock Industries of Thailand.** Page 29.

APHCA (Animal Production and Health Commission for Asia and the Pacific), FAO. (2002). **The Livestock industries of Thailand.** RAP publication No. 2002/23

Arranz, J. J., Bayon, Y., and Primitivo, S. (1998). Genetic relationships among Spanish sheep using microsatellites. **Anim. Genet**. 29: 435-440.

Ayala, F.J. (1982). Population and Evolutionary Genetics: A Primer. **The Benjamin Cummings Pub. Co. Inc,** California, pp: 268.

Ayala, F.J., Tracey, M.L., Hedgecock, D., and Richmond, R. (1974). Genetic differentiation during the speciation process in *Drosophila*. **Evolution**. 28: 576-592.

Baumung, B. R., Simianer, H., and Hoffmann, I. (2004). Genetic diversity studies in farm animals – a survey. **J. Anim. Breed. Genet.** 121: 361-373.

Behl, R., Sheoran, N., Behl, J., and Vijh, R.K. (2006). Genetic analysis of Ankamali pigs of India using microsatellite markers and their comparison with other domesticated Indian pig types. **J. Anim. Breed. Genet.** ISSN 0931-2668.

Botstein, D., White, R.L., and Skolnick, M. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. **American Journal of Human Genetics.** 32: 314-331.

Bradley, D.G., MacHugh, D.E., Cunningham, P., and Loftus, R.T. (1996). Mitochondrial diversity and the origins of African and European cattle. **Proceedings of the National Academy of Sciences.** USA, 93: 5131–5.

Brown, W.M., George, M.J. and Wilson, A.C. (1979). Rapid evolution of mitochondrial DNA. **Proceedings of the National Academy of Sciences of USA**. 76: 1967-71.

Brown, W.M. (1983). Evolution of animal mitochondrial DNAs. **Sunderiand MA Sinauer.** 62-78.

Brown, T. A. (1994). DNA Sequencing. **Oxford University Press.** P2-74.

Cavalli-Sforza, L.L., and Edwards, A.W.F. (1967). Phylogenetic analysis : models and estimation procedure. **Evolution.** 21: 550-570.

Chaiwatanasin, W., Somchai, C., Srisuwan, C., Neramit, S., and Sompoch, T. (2002). Genetic Diversity of Native Pig in Thailand Using Microsatellite Analysis. **Kasetsart J.** (Nat. Sci.) 36: 133 -137.

Chhabra, A.K., Gaur, G.K., Ahlawat, S.P.S., andPaul, S. (1999). Inheritance of carcass traits in desi pigs. **Indian Vet. J.** 76: 403–407.

Dorji, T., Hanotte, O., Arbenz, M., Rege, O., and Roder, W. (2003). Genetic diversity of indigenous cattle populations in Bhutan: implications for conservation. **Asian-Aust. J. Anim. Sci.** 16: 946-951.

Efron, B. (1982). The Jackknife, the Bootstrap and Other Resampling Plans. Philadelphia, PA: Soc. **Ind. Appl. Math.**

Fan, B., Wang, Z. G., Wang, Li., Zhao, X. L., Liu, B., Zhao, S. H., Li. M., Chen, M. H., Xiong, T. A., and Li, K. (2002). Genetic variation analysis within and among Chinese indigenous swine populations using microsatellite markers. **Anim. Genet.** 33: 422-427.

Fang, M., X. Hu, T. Jiang, M. Braunschweig, L. Hu, Z. Du, J. Feng, Q. Zhang, C. Wu, and N. Li. (2005). The phylogeny of Chinese indigenous pig breeds inferred from microsatellite markers. Anim. Genet. 36: 7-13.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. **Evolution** 39: 783–791.

Felsenstein, J. (1988). Phylogenies from molecular sequences: inference and reliability. **Annu. Rev. Genet.** 22: 52–65.

Fitch, W.M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. **Sys. Zool.** 20: 406–416.

Gaur, G.K., Chhabra, A.K., and Paul, S. (1997). Growth intensity of indigenous pigs from birth to slaughter age. **Indian J. Anim. Sci.** 67: 344–346.

Genetic diversity and condition factor: a significant relationship in Flemish but not in German populations of the European bullhead (Cottus gobio L.).**Heredity**. 89: 280- 287.

Geldermann, and valle-zarate. (2004). Genetic diversity and distance of Vietnamese and European pig breeds analysed with microsatellite loci. **University of Hohenheim, Diss.**

Giuffra, E., Kijas, J. M. H., Armager, V., Carlborg, O., Jeon, J.T., and Andersson, L. (2000). The origin of the domestic pig: independent domestication and subsequent introgression. **Genetics.** 154: 1785-91.

Halliburton, R. (2004). Introduction to Population Genetics. **Pearson Education International, New Jersey**. pp: 650.

Hammond, K., and Leitch, H. W. (1998). Genetic resources and the global programme for their management. In: **The Genetics of the Pig** (ed. by M.F. Rothschild & A. Ruvinsky), pp: 405–26. CAB International, New York.

Hanotte, O., Bradley, D. G., Ochieng, J. W., Verjee, Y., Hill, E. W., and Rege, J. E. O. (2002). African pastoralism: genetic imprints of origin and migrations. **Science.** 296: 336-339.

Hartigan, J.A. (1973). Minimum evolution fits to a given tree. **Biometrics**. 29:53–65.

Hedrick, P.W. (1999). Genetics of population, **2nd ed. Jones and Bartlett Publishers. Sudbury,** Massachusetts. pp: 553.

Hedrick, P. W. (2000). Genetics of Populations. J**ones and Bartlett Publishers Inc., Boston.** pp: 629.

Hendy, M.D, and Charleston, M. A. (1993). Hadamard conjugation: a versatile tool for modelling nucleotide sequence evolution. **New Zealand J. Bot.** 31: 231–237.

Hendy, M.D, and Penny, D. (1989). A framework for the quantitative study of evolutionary trees. **Syst. Zool.** 38: 297–309.

Hendy, M. D., Penny, D., and Steel, M.A. (1994). A discrete Fourier analysis of evolutionary trees. **Proc. Natl. Acad. Sci.** *USA* 91: 3339–3343.

Hoelzel, A. R. (1999). Impacts of population bottleneck on genetic variation and the importance of life history; a case study of the northern elephant seal. **Mol. Genet. Animal Ecol.** 68: 23-39.

Kijas J.M.H., Wales, R., and Tornsten, A. (1998). Melanocortin receptor 1 (MC1R) mutations and coat color in the pig. **Genetics.** 150: 1177–85.

Kim, T. H., Kim, K. S., Choi, B. H., Yoon, D. H., Jang, G. W., Lee, K. T., Chung, H. Y., Lee, H. Y., Park, H. S., and Lee, J. W. (2005). Genetic structure of pig breeds from Korea and China using microsatellite loci analysis. **J. Anim. Sci.** 83: 2255–2263.

Kumar, S., Singh, S.K., Singh, R.L., Sharma, B.D., Dubey, C.B., andVerma, S.S. (1990). Effect of genetic and non-genetic factors on body weight, efficiency of food utilization, reproductive performance and survivability in land race, Desi and their halfbreds. **Indian J. Anim. Sci.** 60: 1219–1223.

Kyung, S. K., Yeo, J. S., and Kim, J. W. (2002). Assesment of genetic diversity of Korean native pigs using AFLP markers. **Genes Genet. Syst.** 77: 361-368.

Lemus-Flores, C., Ulloa-Arvizu, R., Ramos-Kuri, M., Estrada, F. J., and Alonso, R. A. (2001). Genetic analysis of Mexican hairless pig populations. **J. Anim. Sci.** 79: 3021–3026.

Li, K., Chen, Y., Moran, C., Fan, B., Zhao, S., and Peng, Z. (2000). Analysis of diversity and genetic relationships between four Chinese indigenous pig breeds and one Australian commercial pig breed. **Animal Genetics.** 31: 322–325.

Luikart, G., Gielly, L., Excoffier, L., Vigne, J.D., Bouvet, J., and Taberlet, P. (2001). Multiple maternal origins and weak phylogeographic structure in domestic goats. **Proceedings of the National Academy of Sciences.** 98: 5927–32.

MacHugh, D. E., Shriver, M. D., Loftus, T. T., Cunningham, P., and Bradley, D. G. (1997). Microsatellite DNA variation and the evolution, domestication and phylogeography of Taurine and Zebu cattle (Bos taurus and Bos indicus). **Genetics.** 146: 1071–1086.

MacKay, S. L. D., Oliver, P. D., and Laipis, P. J. (1986). Template - directed arrest of mammalian mitochondrial DNA synthesis. **Mol. Cell.** 6: 1261-126.

Martınez, A. M., Delgado, J. V., Rodero, A., and Vega-Pla, L. (2000). Genetic structure of the Iberian pig breed using microsatellites. **Anim. Genet.** 31: 295–301.

Nei, M. (1972). Genetic distance between populations. Amer. Nat. 106: 283-292. *Cited* Hedrick, P.W. 1999. Genetics of population, 2nd ed. **Jones and Bartlett Publishers.** Sudbury, Massachusetts. pp: 553.

Nei, M. (1987). Molecular Evolutionary Genetics. New York: **Columbia Univ. Press**

Nei, M. and Kumar, S. (2000). Molecular Evolution and Phylogenetics. **Oxford University Press,** New York. Pp: 333.

Okumura, N., Kurosawa, Y., and Kobayashi, E. (2001). Genetic relationship amongst the major non-coding regions of mitochondrial DNAs in wild boars and several breeds of domesticated pigs. **Animal Genetics.** 32: 139–47.

Randi, E., Lucchini, V., and Diong, C.H. (1996). Evolutionary genetics of the suiformes as reconstructed using mtDNA sequencing. **Journal of Mammalian Evolution.** 3: 163-94.

SanCristobal, M., Chevalet, C. *et al.* (2002). Genetic diversity in pigs – preliminary results on 58 European breeds and lines. **7th World Congress on Genetics Applied to Livestock Production.** Montpellier, France.

Shaklee, J.B., Tamaru, C.S., and Waples, R. (1982). Speciation and evolution of marine fishes studied by the electrophoretic analysis of proteins. **Pacific Science**. 36 (2): 141-157.

Tomowo, O., *et al.* (2000). Molecular Phylogenetic Analysis of the Wild Boars and Native Domestic Pigs in Lao. **Rep. Soc. Res. Native Livestock**. 18: 149-158.

Vernesi, C., *et al.* (2003). The genetic impact of demographic decline and reintroduction in the wild boar (*Sus scrofa*): Amicrosatellite analysis. **Molecular Ecology**. 12: 585-595.

Wallace, D. C. (1993). Mitochondrial disease : genotype versus phenotype. **TIG**. 9 (4): 128-133.

Weber, J.L. (1990). Human DNA Polymorphisms based on length variations in simple sequence tandem repeats. Page 159-181. In; KE Davs and SM Tilghman (eds.), **Genome Analysis.** Vol 1: Genetic and hysical Mapping. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Wolstenholme, D.R. (1992). Animal mitochondrial DNA: structure and evolution. **International Review of Cytology**. 141: 173-215.

Yaetsu, K., Tanaka, K., Nishibori, M., Yamamoto, Y., Namicawa, T., and Bouahom, B. (2000). **Rep. Soc. Res. Native Livestock**. 18: 137-139.

Zardoya, R., and Meyer, A. (1996). Phylogenetic performance of mitochondrial protein-coding genes in resolving relationships among vertebrates. **Mol Biol Evol**. 13: 933–942.

Zhang, G. X., *et al.* (2003). Genetic Diversity of Microsatellite Loci in Fifty-six Chinese Native Pig Breeds. **Acta Genetica Sinica.** 30(3) 225-233.

CHAPTER III

GENETIC DIVERSITY OF THAI INDIGENOUS PIG POPULATIONS, WILD BOARS AND A CHINESE QIANBEI BLACK PIG POPULATION BASED ON MICROSATELLITES

3.1 Abstract

To understand molecular genetic characteristics of Thai indigenous pig populations, the genetic relationships of four populations including two Thai pigs (Northeast Thai pigs and South Thai pigs), a wild boar population living in Thailand, and Chinese Qianbei Black pigs from Guizhou province of China were characterized. A total of 15 microsatellite markers recommended by FAO/ISAG were employed but 12 microsatellite loci could obtain PCR products. The results indicated that all loci were polymorphic and the total observed number of alleles per locus varied from 5 to 17 in all populations. The mean value of all loci was 9.08. The mean number of alleles per locus in single population ranged from 6.5 to 10.75, the average effective number of alleles was from 5.19 to 7.09. The value of average Polymorphism Information Content (PIC) for single population ranged from 0.77(WB) to 0.82(NT). The expected heterozygosity of all populations ranged from 0.69 to 0.96, the average expected heterozygosity within populations was from 0.84 to 0.87. All populations were in Hardy-Winberg equilibrium, but for 7 of 12 loci were significantly deviated

from HWE (P< 0.05). The disequilibrium might be cause by genotyping error, null allele non-random sampling or inbreeding. Hardy-Winberg test has shown no heterozygote excess in all loci in all populations. The mean F_{ST}, a measure of genetic divergence among the subpopulations, ranged from 0.047 to 0.113, of all loci indicated that 91.1% of the genetic variation was caused by the differences among individuals and only 8.9% was due to the differentiation among populations. A UPGMA tree based on Nei's D_A standard genetic distances indicated that Chinese Qianbei Black pigs (CQB) and two Thai indigenous pig populations (NT, ST) were clustered into the same branches with a 100% of bootstrap support value, whereas wild boars (WB) were clustered into another branch. From current results, Thai native pig population might have the same origin as pigs of south or southwest China. These findings can be used as genetic information and further genetic improvement of Thai indigenous pigs.

3.2 Introduction

Thai indigenous pig populations are mainly distributed in central and northeast regions of Thailand, which has more than 50 percent of Thailand's pig population (APHCA, 2002). In the past decades, a large number of native pigs in Thailand have been gradually disappearing due to the increase of introduced species. The conservation of genetic diversity for Thai native pig populations has become more and more important.

Microsatellites are widely used to study the genetic diversity in plants and animals because of the high information content of the genetic data compared to other molecular markers such as RFLP、RAPD and so forth. On the other hand, identifying

animal genetic diversity using microsatellite makers is more precise and more effective than that using traditional methods such as cytogenetic and biochemical methods (Baumung et al., 2004). Because the individual genotypes can be obtained with the aid of the property of polymorphism and co-dominance of microsatellite DNA. The allele frequencies mean heterozygosity can be calculated. The genetic distance can be computed and dendrogram can also be analyzed.

Previous studies on genetic variations in Thai pigs mainly based on morphological characteristics, a little information was acquired based on molecular markers. Chaiwatanasin (2002) reported study on genetic analysis of the Thailand indigenous pig populations using microsatellite markers. However, samples for this research were taken only from the northeast and north of Thailand, samples from south of Thailand were not used. The study on polymorphism of serological protein in Thai native pigs conducted by Tanaka (1974), was not able to find significant differences among three types of Thai native pigs. Any reports of analyses on the genetic diversity based on microsatellites among indigenous pigs from the northeast and south of Thailand, wild boar and Chinese domestic pigs. The main objective of this experiment was to study and document genetic diversity among these pig populations.

3.3 Materials and Methods

3.3.1 Determination of optimum tissues for appropriate amplification of microsatellites.

3.3.1.1 Samples

Three indigenous Thai pigs which were raised at Suranaree University of Technology farm (SUT farm) were used as the sampling pigs. Hair roots were collected from the rear quarters of pigs after sterilizing with 80 % alcohol. Each sample was separated into two 1.5ml centrifuge tubes, one tube contained 100 hair roots, another one contained about 200 hair roots, 5ml of blood sample was withdrawn from the same pigs at precaval vein, blood samples were collected in the presence of EDTA and kept at -20℃ until use (Table 3.1).

Table 3. 1 Collection and grouped method from pig blood and hair root samples

Number	**blood samples**	**hair root samples**
Pig I	B1(5 ml)	H1(100)
Pig II	B2(5 ml)	H2(200)
Pig III	B3 (5ml)	H3(200)

3.3.1.2 DNA extraction

Wizard Genomic DNA Purification Kit was employed to extract DNA in this experiment with a little bit of decoration for hair roots samples, An addition of Proteinase K (15 μg/ml for each sample) was applied in order to enhance digestive ability to hair tissue. The whole process for DNA extraction is described below: For 100 hair root samples, after adding 200 μL of Nucleic lysis solution, 10μL of proteinase K was added for each tube. After that samples were incubated at 55℃ for 24 hours. Then 1μL of RNase solution was added, mixed by inverting tubes 30 minutes at 37℃. After cooling the samples at room temperature for 5 minutes, 67 μL of protein precipitation solution was added. Vortex at high speed for 20 seconds, then the sample was chilled on ice for 5 minutes. The sample was run on centrifuge at the rate of 12500 rpm for 15 minutes, supernatant was discarded and 200 μL of 70% of

ethanol was added to wash, then centrifuge again at 12500 rpm for 15 minutes. For 200 hair root samples, the dose for added reagents were double that of the 100 hair root samples. Finally, it was air-dried for 15 minutes, 30 μL of SDW was add and kept at 4°C.

Table 3.2 Major reagents and amount for DNA extracting used in this experiment

	Blood (350 μ L)	**Hair roots (100)**	**Hair roots (200)**
Cell Lysis Solution	1050	no	no
Nuclei Lysis Solution	350	200	400
0.5 M EDTA	84	48	96
Proteinase K	5	10	20
RNase A	1.5	1.0	2.0
Protein Precipitation	118	67	135
Isoprepoaol	350	200	400

For DNA extraction of blood samples, 350 μL of whole blood were taken from a total of 5 ml whole blood samples. The major reagents and additional amount are listed in Table 3.2 Only 5 μL of proteinase K was added for blood sample.

3.3.1.3 Primers and PCR

Two pairs of microsatellite primers (S0225, S0227) were used for preliminary study of suitable DNA template for PCR conditions. PCR was performed according to the following condition: denaturing at 95°C for 5 min, and then followed

by 35 cycles at 95°C for 30 sec and 53-55°C for 30 sec and followed by 72°C for 30 seconds. 72°C extension for 5 minutes. In order to check whether PCR products acquired from the hair root samples DNA sources can be used for polyacrylamide gel electrophoresis (PAGE) or not, 21 of PCR products from DNA amplification of 21 Thai indigenous pig hair root samples were used to run PAGE.

Table 3.3 Primer sequences and amplification conditions of 2 pairs of microsatellites

Microsatellites	primer sequences (5'-3')	Mg^{2+} (mmol/L)	Ann. temp. (℃)
S0225	GCTAATGCCAGAGAAATGCAGA(Forward)	4.0	53
	CAGGTGGAAAGAATGGAATGAA(Reverse)		
S0227	GATCCATTTATAATTTTAGCACAAAGT(Forward)	1.5	55
	GCATGGTGTGATGCTATGTCAAGC(Reverse)		

3.3.2 Determination of optimum DNA template concentrations for appropriate amplification of microsatellites

Every sample including hair root samples and blood samples was diluted into three different of DNA concentrations 1 ng/μL, 2.5 ng/μL, 5.0 ng/μL. and then PCR was performed in a 10 μL final volume with 1 μL of 10 × buffer, 0.8 μL of 2.5 mM dNTP, 0.6 μL of 20 mM $MgCl_2$, 1 μL of 10 pmol of each primer, and 0.05 μL of *Taq* DNA enzyme, and 1 μL of DNA template. Thermal cycling conditions included an initial denaturing for 5 min at 95°C, followed by 35 cycles of 30 sec at 95°C, 30 sec at annealing temperature 53-55°C, 30 sec at 72°C, and a final extension step of 72°C for 5 min.

3.3.3 Sampling collection for North Thai pigs (NT) and wild boars (WB)

3.3.3.1 Sampling site

Six provinces including 11 districts in northeast Thailand were used as sampling sites. Photos for North Thai pigs are shown in Figures 3.1 to 3.4, and the photos for Wild Boars are shown in Figures 3.5 and 3.6. The numbers of sampling pigs for each province are listed in Table 3.4. Most of the samples came from Sakon Nakhon and Loei provinces; while a small amount of sampling pigs were taken from Sisaket province. The sampling size depends on the numbers of reared pigs.

Table 3.4 Information for sampling site

Date	Province/Sampling site	District/Sampling site	No. of sampling pigs	
			Native pigs	Wild boars
10-12/06/2006	Sakon Nakhon	Good Bahk;	10	3
23-25/06/2006		Morng;	1	
		Tow Ngaoy	1	2
23-25/06/200	Nakon Panom	Nah wah;	4	1
		Morng;	1	1
30/06/2006	Loei	Chieng karn;	7	
02/07/2006		Tha li;	4	
		Wang Saphong;	1	
14-16/06/2006	Mukdahan; Si Saket; Surin	Wan Yai; Morng; PhanomDongPak	11 2 8	
20/07/2006 ～7/06/2006	Chinese Qianbei Black pigs	Zunyi (Guizhou, China);	20	
Sum		Northestern north	50	7

3.3.3.2 Breed identification

For Hailum, major parts of body are black, with the abdomen, the four limbs and pettitoes being white. It has the small and up-right ears, longer nose bending upwards and the small buttock. Plus, it has the weak leg with a little bit curves. Speed of growth is quick; this type of pig grows more quickly than other relative types in Thailand. The body weight of adult pigs may amount to 112 ~ 120 kg.

For Mukuai, its body shape is similar to Hailum, but many more wrinkles and larger ears than Hailum pig. The whole body is covered by black hair.

Murad, pigs are smaller than Hailum and Mukuai pig, and the whole body is covered by black hair.

Figure 3.1 Sample 1(2L) for North Thai pig

Figure 3.2 Sample 2(4L) for North Thai pig

Figure 3.3 Sample 3(5NP) for North Thai pig

Figure 3.4 Sample 4(2MD) for North Thai pig

Figure 3.5 Sample 5(2NP) for Wild Boar

Figure 3.6 Sample 6(8SN) for Wild Boar

3.3.3.3 Sampling method

As mentioned in chapter Π, Indigenous pigs have been fed by farmers in their villages; no special comprehensive farms were used for feeding them. Normally, each farmer's family has two or three native pigs. Therefore, sample collection had to be conducted from one farmer's house to another, from one village to another. The route of sampling was from Sakon Nakhon province to Nakon Phanom, then to Loei, Surin, Mukdahan and Sisaket province. In most cases, the pigs were more than two

years old. After sterilizing the rear-back skin using 80% alcohol, 100-200 hair roots containing hair follicles were taken out and put into 1.5ml centrifuge tube, three tubes were needed for each pig, then kept on ice until transferring them to environment of -20°C. In addition, body size measurement was also performed and recorded before taking the hair roots out. Four indices including body length, body height, circumference, head length were recorded.

3.3.4 Sampling collection for Chinese Qianbei Black pigs (CQB)

3.3.4.1 Sampling site

At Zunyi district, located at north region of Guizhou province (Figure 3.12), China, 20 samples were taken from Qianbei Black pig conservation farm. Samples were taken according to the shape criterion of Qianbei Black pig breed (Figure 3.7 to Figure 3.10).

3.3.4.2 Morphological characteristics

Qianbei Black pigs are mainly distributed in northeast Guizhou province, China. we can find this type of pig in more than 20 counties of this province. The whole body is covered by black hair (Figure 3.7). The size of the head is moderate, with small and up-right ears, a longer mouth, many more forehead wrinkles than other breeds in Guizhou. The length of neck is moderate, the chest is slightly narrow and deep, and the abdomen hangs down greatly. The four limbs are healthy and strong, back parts of the body are relatively developed. It has some outstanding productive traits, such as high fertility, adaptation to harsh conditions and poor quality feed, a high dressing percentage and good quality of pork (GAPSC, 1993).

Figure 3.7 Sample 1 for Qianbei Black pig

Figure 3.8 Sample 2 for Qianbei Black pig

Figure 3.9 Sample 3 for Qianbei Black pig

Figure 3.10 Sample 4 for Qianbei Black pig

3.3.5 Sample collection for South Thai pigs (ST)

Twenty two Thai native pig samples from south of Thailand were collected from Nakon Si Thammarat province.

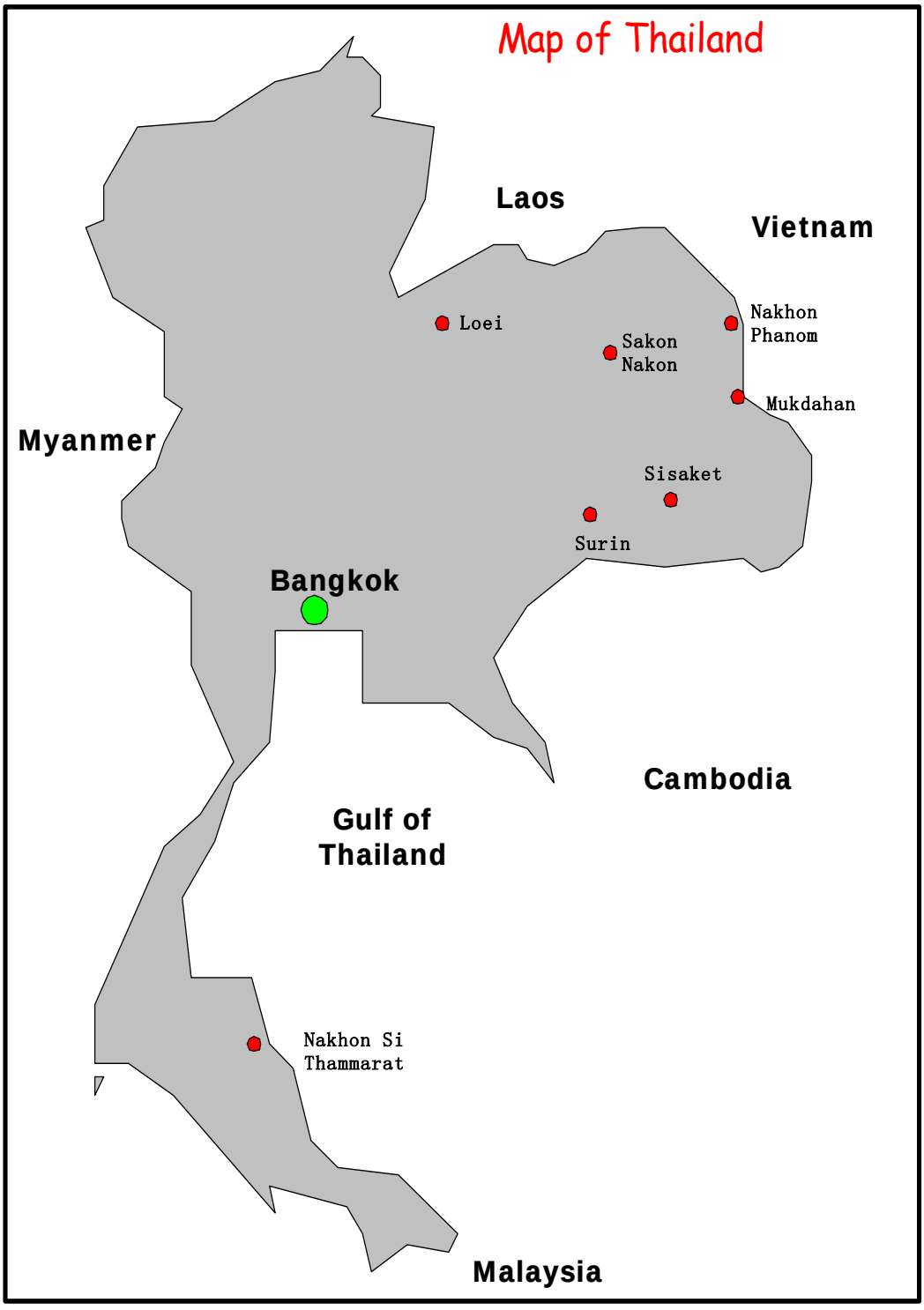

Figure 3.11 Sampling sites for indigenous pig in Thailand

Map of Guizhou Province, China

Figure 3.12 Sampling site for Qianbei Black pigs in Zunyi, Guizhou

All samples came from adult sows or boars with accordant appearances who were more than two years old. Body sizes were measured and recorded, and then hair roots were pulled out from the back of pigs after sterilizing the root using 80 % alcohol. Three repeated hair root samples were put into 1.5ml centrifuge tubes; each tube contained more than 100 hair roots.

3.3.6 Microsatellite markers

Of all 27 pairs of microsatellite primers in swine recommended by FAO/ISIG in 2004, fifteen primers were selected to amplify microsatellite DNA in this experiment, which are most frequently used in the researches of genetic diversity in pigs, the information for these fifteen primers were listed in Table 3.5. Each marker is considered to locate at different chromosomes; consideration of marker selection depends on: (1) Genomic location; (2) Allele number; and (3) Ease of scoring.

Table 3.5 Information of 15 pairs of microsatellite primers applied in this experiment

Primer	Sequence of primers (5'-3')	Chr.	Ann.Temp. /$Mgcl_2$ (mM)	Size allele(bp)
S0227	GATCCATTTATAATTTTAGCACAAAGT	4	55 / 4.0	231-256
	GCATGGTGTGATGCTATGTCAAGC			
S0090	CCAAGACTGCCTTGTAGGTGAATA	12	58 / 1.5	244-251
	GCTATCAAGTATTGTACCATTAGG			
S0226	GCACTTTTAACTTTCATGATACTCC	2q	55 / 4.0	181-105
	GGTTAAACTTTTNCCCCAATACA			
S0005	TCCTTCCCTCCTGGTAACTA	5	58 / 1.5	205-248
	GCACTTCCTGATTCTGGGTA			
S0068	AGTGGTCTCTCTCCCTCTTGCT	13	62 / 1.5	211-260
	CCTTCAACCTTTGAGCAAGAAC			
S0225	GCTAATGCCAGAGAAATGCAGA	8	55 / 4.0	170-196
	CAGGTGGAAAGAATGGAATGAA			
S0155	TGTTCTCTGTTTCTCCTCTGTTTG	1q	55 / 1.5	150-166
	AAAGTGGAAAGAGTCAATGGCTAT			
SW122	TTGTCTTTTTATTTTGCTTTTGG	6	58 / 1.5	110-122
	CAAAAAAGGCAAAAGATTGACA			
S0355	TCTGGCTCCTACACTCCTTCTTGATG	15	55 / 4.0	243-277
	TTGGGTGGGTGCTGAAAAATAGGa			
S0386	TCCTGGGTCTTATTTTCTA	11	48 / 3.0	15-174
	TTTTTATCTCCAACAGTAT			
SW911	CTCAGTTCTTTGGGACTGAACC	9	60 / 1.5	153-177
	CATCTGTGGAAAAAAAAAGCC			
SW24	TGGGTTGAAAGATTTCCCAA	2p	58 / 1.5	96-115
	GGAGTCAGTACTTTGGCTTGA			
SW632	ATCAGAACAGTGCGCCGT	7	58 / 1.5	159-180
	TTTGAAAATGGGGTGTTTCC			
SW857	AGAAATTAGTGCCTCAAATTGG	14	58 / 1.5	144-160
	AAACCATTAAGTCCCTAGCAAA			
S0002	GAAGCCCAAAGAGACAACTGC	3q	62 / 1.5	19o-216
	GTTCTTTACCCACTGAGCCA			

Applied from ISAG/FAO, 2004

3.3.7 PCR and Polyacrylamide electrophoresis

To detect polymorphism, PCR were performed in 10 μL reaction mixture containing 2.5～5.0 ng of template DNA, 10×buffer, 2.5 mM each of dNTP mixture, 1.5～4.0 mM $MgCl_2$, 10 pmol primer and 0.25 unit of *Taq* DNA polymerase (Fermentas, USA). The amplification was performed in iCycler PCR system (BIO-RAD, USA) with an initial cycle at 95°C for 5 min followed by 35 cycles at 95°C for 30 sec and 48～62°C for 45 sec and followed by 72°C for 30 sec. 72°C extension for 5 min. PCR reactions were performed according to recommended annealing temperatures and concentrates of $MgCl_2$ with suitable adjustments so as to acquire ideal PCR products for running polyacrylamide gel electrophoresis (PAGE).

Three microliters of denatured PCR products were loaded into a 6% denaturing polylamide sequencing gel according to the Protocol established by Promega Corporation. Major operative steps include (1) Glass plate preparation; (2) Polyacrylamide gel preparation; (3) Electrophoresis. Molecular marker 'Ph1×174/Hinf1' and sequencing makers 'M13' ladder were loaded into the middle of each panel gel. Preparations for M13 ladder solution, silver staining solution according to the methods descried by Promega Corporation. Scoring of microsatellite genotypes is straightforward.

3.3.8 Data analysis

The program CONVERT version 1.31 (Glaubitz, 2005) was applied to convert diploid genotypic data files into formats for GENEPOP version 3.4, (Raymond and Rousset, 1995). Numbers of homozygotes and heterozygotes (including expected and observed) and Hardy-Weinberg equilibrium (*HWE*) test were calculated using GENEPOP; numbers of alleles per locus (*No*), effective number of

alleles (*Ne*), expected (H_E) and observed heterozgosity(H_O), allele frequencies, Polymorphism Information Content (*PIC*) values were calculated using POPGENE version 1.31(Yeh et al. 1997). Observed number of alleles and Effective number of alleles were calculated according to Kimura and Crow (1964). Expected homozygosity and heterozygosity were computed according to Levene (1949); Nei's expected heterozygosity was computed according to Nei's (1973). The exact Hardy-Weinberg equilibrium (HWE) was carried out for each locus in each population based on the alternative hypothesis with heterozygosity deficiency or excess. The length of the Markov chain was set to 1,000 iterations per batch for 300 batches and the memorization number was 1,000.

An application 'MICRO-CHECKER' (Shipley, 2003) was used to check the microsatellite data for null alleles and scoring errors. The application uses a Monte Carlo simulation (bootstrap) method to generate expected homozygote and heterozygote allele size difference frequencies. The Hardy-Weinberg theory of equilibrium was used to calculate expected allele frequencies and the frequency of any null alleles detected.

Nei's standard genetic distance (Nei's, 1972) among four pig populations were calculated using a computer package PHYLIP version 3.67 (Felsenstein, 1993). Considering that a small number of individuals, Nei's unbiased genetic distance (Nei 1978) were computed using MICROSAT version 1.5b (Minch, 1998) as well. An unrooted phylogenetic tree was also constructed using UPGMA method based on Nei's unbiased genetic distance using PHYLIP veision 3.67. Bootstraps of 1000 replicates were performed in order to test the robustness of tree topology (Efron et al., 1996).

3.4 Results

3.4.1 DNA quality

Results of 0.7% of agarose gel showed no obvious differences of quality between hair roots samples and blood samples (Lane 4～9 in Figure 3.13). Comparing DNA quality among blood samples (Lane 1～3), Lane 2 appeared obvious tail band, which means there were more DNA fragments. Comparing DNA quality between 100 hair roots(Lane 4～6) and 200 hair roots (Lane 7～9), no significant differences could be found.

OD values of 260 nm wavelength were measured and the results indicated that DNA concentrations were different but the same volume of sampling bloods were used(Table 3.6). DNA concentration from 100 hair roots was 480 ng/μL, while DNA concentration from 200 hair roots was only 380 ng/μL. This suggests that the number of hair roots was not directly relevant to DNA concentrations.

M 1 2 3 4 5 6 7 8 9

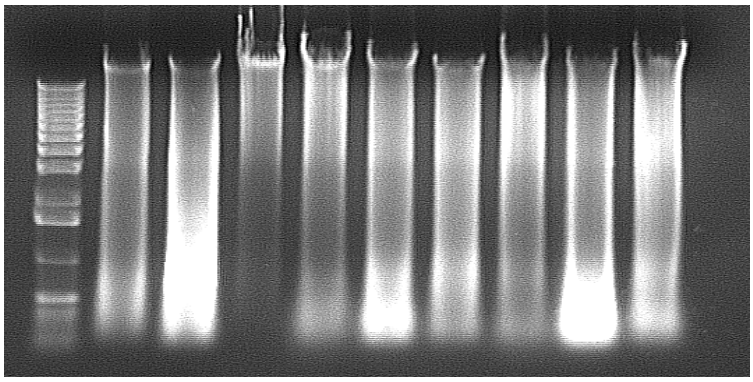

Figure 3.13 The results of 0.7% agarose gel electrophoresis of DNA from blood and hair root samples.

M: DNA marker; Lane1:B1; Lane2:B2; Lane4:H1 (100); Lane7:H2 (200); Lane8:H3 (200)

Table 3.6 OD values of sampling DNA

OD_{260} Value	**ABS**	**Concentration**
B1	0.009	90 ng/μL
B2	0.016	160 /μL
H1	0.048	480 ng/μL
H2	0.038	380 ng/μL
H3	0.117	1170 ng/μL

Equation for calculating DNA concentration: DNA concentration= OD_{260} Value×50ng/μL×200

3.4. 2 PCR condition and DNA template concentrations

PCR were performed to check DNA quality from hair roots, results on microsatellite loci S0225 indicated that most of samples could acquire clear bands except for H1 (Lane 1: 1 ng/μL) and H2 (Lane 4: 1 ng/μL). This means that 1 ng/μL of DNA template concentration from hair roots was not enough for PCR

amplification. Similar bands could be observed in DNA template concentration 2.5 ng/μL and 5.0 ng/μL. In particular, three different DNA concentrations (1 ng/μL, 2.5 ng/μL, and 5 ng/μL) in H3 produced more intensive bands. Comparing the PCR results from blood samples B1（Lane 10～12）and B2 (Lane 13～15), clear bands could be observed. The differences between blood samples and hair root samples were significant. Figure 3.15 indicated PCR amplification result on microsatellite loci S0227, a similar result could be viewed, H1（1 ng/μL）H2(1 ng/μL)，H3(1 ng/μL，5 ng/μL)，B2(1 ng/μL) produced weak bands, but 2.5 ng/μL and 5 ng/μL of DNA concentrations had more intensive bands. Similar results could be found compared with PCR products from microsatellite loci S0225 (Figure 3.14).

To check if DNA taken from hair roots can be used for genetic studies, 21 PCR products on microsatellite loci S0225 and S0227 were used to run 6% polyacrylamide gel electrophoresis (Figure 3.16, Figure 3.17). ϕx174/Hinf Ⅰ marker was used to score the allele size, sequencing marker M13 was used to measure base pair length. Result showed all 21 samples could acquire much clear bands; all allele size could be scored clearly.

M N 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

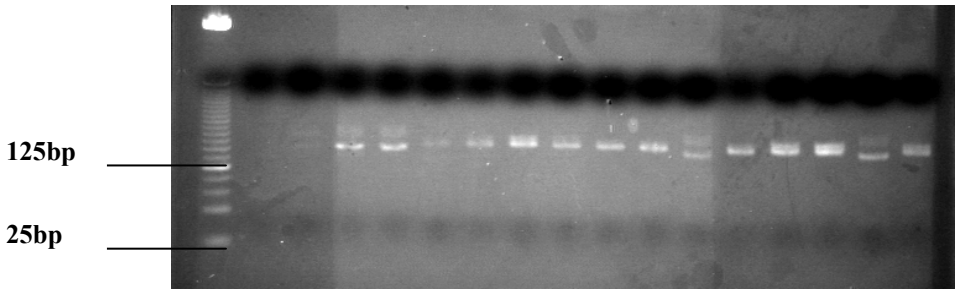

Figure 3.14 PCR results of two kinds of DNA templates sources、three DNA concentrations on microsatellite Loci S0225

M: 25bp DNA ladder; N: Negative control (No DNA template);

Lane 1～3: H1 (1, 2.5, 5 ng/μL; DNA from 100 hairs of Pig I);

Lane 4～6: H2 (1, 2.5, 5 ng/μL; DNA from 200 hairs of Pig II);

Lane 7～9: H3 (1, 2.5, 5 ng/μL; DNA from 200 hairs of Pig III);

Lane 10～12: B1 (1, 2.5, 5 ng/μL; DNA from blood of Pig I);

Lane13～15: B2 (1, 2.5, 5 ng/μL; Genomic DNA from Blood of Pig II)

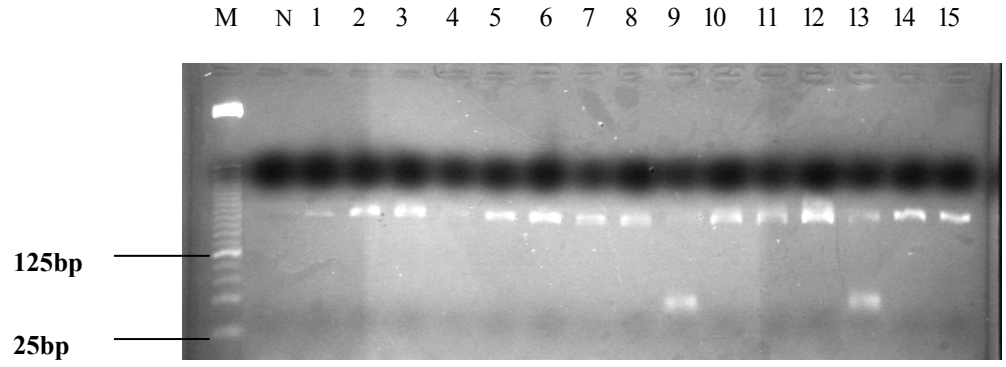

Figure 3.15 PCR results of two kinds of DNA templates sources、three DNA concentrations on microsatellite Loci S0227

M: 25bp DNA ladder; N: Negative control (No DNA template);

Lane1～3：H1 (1, 2.5, 5 ng/μL; DNA from 100 hairs of Pig I);

Lane 4～6: H2 (1, 2.5, 5 ng/μL; DNA from 200 hairs of Pig II);

Lane 7～9: H3(1, 2.5, 5 ng/μL; DNA from 200 hairs of Pig III)；

Lane10～12: B1 (1, 2.5, 5 ng/μL; DNA from blood of Pig I)；

Lane 13～15: B2 (1, 2.5, 5 ng/μL; Genomic DNA from Blood of Pig II)

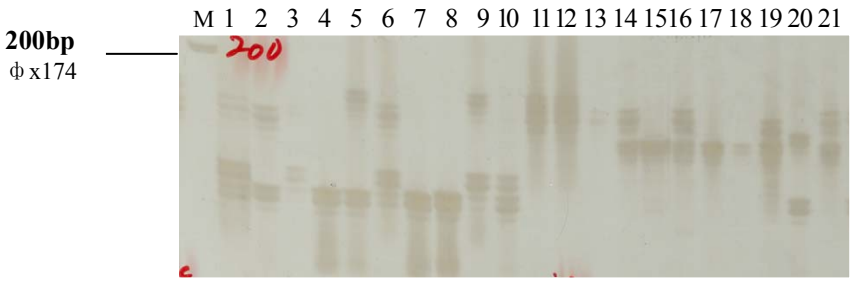

Figure 3.16 PAGE results on microsatellite S0225 using DNA from hair roots in 21 North Thai pigs

(M: ϕx174 Marker; From Lane 1～21: 21 Samples from North Thai pigs:1 SN, 4 SN, 1 NP, 4 NP, 5 NP, 5 SN, 7 SN, 1 L, 2 L, 3 L, 4 L, 5 L, 6 L, 1 U, 2 U, 3 U, 1 MD, 2 MD, 3 MD, 1 SS, 2 SS)

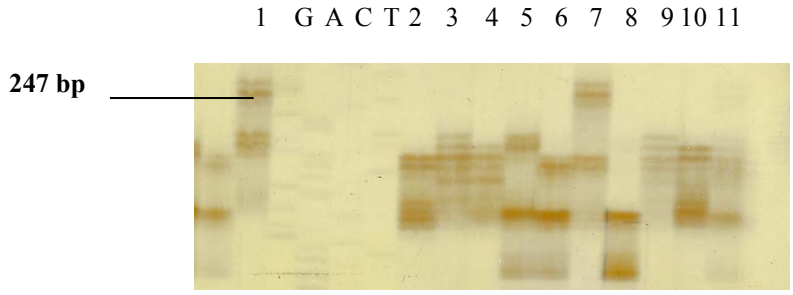

Figure 3.17 PAGE results on microsatellite S0227 using DNA from hair roots in South Thai pigs

(G, A, C, T: Sequencing Markers)

From Lane 1～11: Samples from South Thai pigs: S6, S7, S8, S9, S10, S11, S12, S13, S14, S15, S16. respectively)

3.4.3 Genetic variations within populations

Monte Carlo simulation method by generating expected homozygote（Gils et al., 1996）and heterozygote allele size using MICRO-CHECKER indicated that total expected homozygotes were 11.8 and total observed homozygotes were 22 on loci S0355. Combined probability for presence of null alleles in all classes was significant (P< 0.001), it means that null alleles may be present at this locus (Appendix 4.14). Total expected homozygotes became 6.17 and total observed homozygotes were 9 after adjusting. No evidence could prove the presence for null alleles at this locus.

Table 3.7 The summary for genetic variations for four populations

Population	**Sample size**	H_O	H_E	***PIC***	F_{IS}	***No***	***Ne***	N_{HWE}
CQB	20	20	0.84	0.79	0.02	9.17	5.97	4
ST	22	22	0.84	0.81	0.14*	9.92	6.30	6
NT	21	21	0.86	0.82	0.16*	10.75	7.09	6
WB	7	7	0.87	0.77	0.23*	6.50	5.19	1

*P <0.05, significant.

Notes CQB: Chinese Qianbei Black pigs; ST: south Thai pigs; NT: northeast Thai pigs; WB: wild boars. H_O : Mean observed heterozygosity; H_E : Mean expected heterozygosity; *No* : observed mean number of alleles; *Ne* : Effective mean number of alleles; F_{IS}: inbreeding coefficient; N_{HWE} : Number of loci not in HWE populations.

As shown in Table 3.7, four pig populations including Chinese Qianbei pigs,

South Thai pigs, Northeast Thai pig and wild boars exhibited a high degree of genetic diversity with mean expected heterozygosities of 0.84, 0.84, 0.86, and 0.87, respectively. Table 3.9 listed genetic variations of four pig populations. All loci were polymorphic and the total observed number of alleles per locus varied from 5 (S0155, S122, S0386 and SW24) to 17 (S0068) in all populations. The mean value of all loci was 9.08. The mean number of alleles per locus in single population ranged from 6.5(WB) to 10.75 (NT), the average effective number of alleles was from 5.19 (WB) to 7.09 (NT). The expected heterozygosity of all populations ranged from 0.69 to 0.96, ST(0.84),while observed heterozygosity for all populations ranged from 0.43 to 1.00, the mean value in single population was 0.68 (WB) to 0.82 (CQB), Chinese Qianbei Black pigs had the highest observed heterozygosity among all populations. The value of average Polymorphism Information Content (*PIC*) for single population ranged from 0.77(WB) to 0.82(NT), indicating that NT has highest *PIC* value.

Significant departures from Hardy-Weinberg equilibrium (when H1 = heterozygote deficit) were observed in 7 of 12 single locus exact tests. S0090 and S0005 deviated from HWE in two populations. The other loci including S0226, S0227, S0355, S0386 and SW24 were in disequilibrium only in one population. (Table 3.12). Hardy-Weinberg equilibrium test (when H1= heterozygote excess) indicated no significances on all loci. Across multiple loci, South Thai pigs, Northeast Thai pigs and wild pig, showed a significant value of inbreeding coefficient (*F*IS) after correction for multiple tests. All populations showed no deviation ($P > 0.05$) from HW equilibrium (Table 3.9). Estimation of exact P-values by the Markov chain method indicated that significant heterozygosity deficits were the main cause for deviation from HWE.

The overall F_{IS} values per locus ranged from -0.1137 (S0355) to 0.2892, average value was 0.09. F_{ST} values ranged from 0.047(S0226) to 0.113 (S0090). The mean F_{ST} value of 0.089 from all loci indicated that 91.1% of the genetic variation was caused by the differences among individuals and 8.9% was due to the differentiation among populations.

Table 3.8 Characterization of the 12 microsatellites analyzed in four pig populations

Locus	F_{IS}	F_{IT}	F_{ST}
S0227	0.2084	0.2806	0.0912*
S0090	0.2611	0.3444	0.1127*
S0226	-0.0464	0.0024	0.0466*
S0005	0.2475	0.2899	0.0563*
S0068	0.0350	0.1264	0.0947*
S0225	0.1238	0.2046	0.0922*
S0155	0.0474	0.1298	0.0865*
SW122	0.0217	0.0969	0.0769*
S0355	-0.0805	0.0376	0.1093*
S0386	0.2892	0.3604	0.1002*
SW911	-0.1137	-0.0120	0.0913*
SW24	0.0959	0.1968	0.1116*
Mean	0.0900	0.1710	0.0890

F_{ST} is measures of the genetic differentiation over subpopulations.

Bonferroni correction (P< 0.05/12= 0.0041) *$P < 0.05$;

3.4.4 Inter-population structures

Genetic distances among four populations are shown in Table 3.14. Nei's standard genetic distances (Nei, 1972) ranged from 0.644 to 1.202. Chinese Qianbei Black pigs (CQB) and wild boars (WB) had the largest distance, while Northeast Thai pigs (NT) and wild boars (WB) had the smallest. CQB and NT, CQB and ST had larger genetic distance than ST and NT, ST and WB, NT and WB as well. Nei's unbiased genetic distances (Nei, 1978) was also measured considering that with a

comparatively smaller pig population, a similar result could be obtained although the absolute values of genetic distances were different from Nei's standard genetic distances.

3.4.5 A phylogenetic tree

A phylogenetic tree of four pig populations was constructed based on Nei's D_A standard genetic distances using UPGMA method (Figure 3.13). Indigenous pigs from northeast Thailand (NT) was grouped into the same branches with indigenous pigs from south Thailand (ST) with a 73% of bootstrap support value. Chinese Qianbei Black pigs (CQB) and two Thai indigenous pig populations (NT, ST) were clustered into the same branches with a 100% of bootstrap support value, whereas wild boars (WB) were clustered into another branch. This result indicated that Chinese Qianbei pigs (CQB) had closer relationship with two Thai indigenous pig populations (NT, ST) than with wild boars (WB).

Table 3.9 Main parameters of genetic variation based on msDNA data in four populations

P	V	Microsatellite loci												M/L
		S0227	**S0090**	**S0226**	**S0005**	**S0068**	**S0225**	**S0155**	**SW122**	**S0355**	**S0386**	**SW911**	**SW24**	
CQB	N	40	30	38	36	34	40	38	38	34	32	30	40	35.83
	No	11	7	11	13	10	8	11	9	8	9	6	7	9.17
	Ne	6.29	5.36	7.60	10.12	5.03	4.94	7.37	5.78	3.19	6.74	4.21	4.97	5.97
	PIC	0.83	0.79	0.86	0.89	0.78	0.77	0.84	0.81	0.65	0.83	0.73	0.77	0.79
	H_E	0.86	0.84	0.89	0.93	0.83	0.82	0.89	0.85	0.71	0.88	0.79	0.82	0.84
	H_O	0.80	0.73	0.95	0.89	0.76	0.85	0.95	0.89	0.65	0.86	0.87	0.60	0.82
	H_O/H_E	0.93	0.87	1.07	0.96	0.92	1.04	1.07	1.05	0.92	0.98	1.10	0.73	0.97
ST	N	40	40	44	38	38	44	42	40	38	38	36	42	40
	No	9	8	13	12	11	6	11	11	9	8	10	11	9.92
	Ne	4.52	6.56	8.05	7.37	6.69	3.72	7.41	7.27	3.94	5.05	7.90	7.17	6.30
	PIC	0.75	0.83	0.82	0.85	0.84	0.70	0.85	0.85	0.76	0.78	0.86	0.85	0.81
	H_E	0.79	0.86	0.85	0.89	0.87	0.74	0.89	0.88	0.77	0.82	0.89	0.88	0.84
	H_O	0.65	0.55	0.73	0.58	0.89	0.64	0.67	0.95	0.68	0.58	0.94	0.71	0.71
	H_O/H_E	0.82	0.64	0.86	0.65	1.02	1.16	0.75	1.08	0.88	0.71	1.06	0.81	0.87

V =Variability; P =Population; M/L = Mean of all loci; *No* =Observed number of alleles; Ne= Effective number of alleles [Kimura and Crow (1964)]; *PIC*= Polymorphism Information Content(Botstein et al.,1980); H_O =observed heterozygosity, H_E = Expected heterozygosity[Levene (1949)];.CQB =Chinese Qianbei Black pigs, ST = South Thai pigs; NT =North Thai pigs, WB =Wild Boars.

Table 3.9 (Continued) Main parameters of genetic variation based on msDNA data in four populations

P	V	Microsatellite loci												M/L
		S0227	**S0090**	**S0226**	**S0005**	**S0068**	**S0225**	**S0155**	**SW122**	**S0355**	**S0386**	**SW911**	**SW24**	
NT	N	42	34	42	40	40	42	42	40	36	34	36	36	38.67
	No	11	7	15	16	17	11	8	7	11	8	10	8	10.75
	Ne	7.67	4.35	8.02	12.5	10.7	7.54	4.64	4.65	8.88	3.11	6.82	6.29	7.09
	PIC	0.86	0.74	0.87	0.91	0.90	0.85	0.76	0.75	0.88	0.65	0.84	0.83	0.82
	H_E	0.89	0.79	0.89	0.94	0.93	0.89	0.80	0.81	0.91	0.69	0.88	0.87	0.86
	H_O	0.71	0.53	0.95	0.60	1.00	0.71	0.67	0.70	0.44	0.53	0.89	0.83	0.71
	H_O/H_E	0.79	0.67	1.07	0.64	1.08	0.79	0.84	0.86	0.48	0.77	1.01	0.95	0.83
WB	N	14	14	14	10	10	10	10	10	14	10	12	10	11.5
	No	6	7	11	8	6	6	5	5	7	5	7	5	6.5
	Ne	4.67	4.9	8.91	7.14	5.56	5.00	3.57	3.84	4.66	3.84	6.00	4.17	5.19
	PIC	0.76	0.77	0.88	0.77	0.79	0.77	0.76	0.70	0.76	0.70	0.81	0.72	077
	H_E	0.85	0.86	0.96	0.96	0.91	0.89	0.80	0.82	0.85	0.82	0.91	0.84	0.87
	H_O	0.43	0.57	1.00	0.60	0.60	0.60	0.80	0.60	1.00	0.20	1.00	0.80	0.68
	H_O/H_E	0.51	0.66	1.04	0.63	0.66	0.67	1.00	0.73	1.17	0.24	1.09	0.95	0.78

V =Variability; P =Population; M/L = Mean of all loci; *No* =Observed number of alleles; Ne= Effective number of alleles [Kimura and Crow (1964)]; *PIC*= Polymorphism Information Content (Botstein et al.,1980); H_O =observed heterozygosity, H_E = Expected heterozygosity[Levene (1949)];.CQB =Chinese Qianbei Black pigs, ST = South Thai pigs; NT =North Thai pigs, WB =Wild Boars.

Table 3.10 Effective number of alleles (*Ne*) and Observed number of alleles (*No*) in four pig populations

Locus	Pop 1 (CQB)			Pop 2 (ST)			Pop3 (NT)			Pop 4 (WB)		
	No	***Ne***	**Smpl Size**	***No***	***Ne***	**Smpl Size**	***No***	***Ne***	**Smpl Size**	***No***	***Ne***	**Smpl Size**
S0227	11.0000	6.2992	40	9.0000	4.5198	40	11.0000	7.6696	42	6.0000	4.6667	14
S0090	7.0000	5.3571	30	8.0000	6.5574	40	7.0000	4.3459	34	7.0000	4.9000	14
S0226	11.0000	7.6000	38	13.0000	6.0500	44	15.0000	8.0182	42	11.0000	8.9091	14
S0005	13.0000	10.1250	36	12.0000	7.3673	38	16.0000	12.5000	40	8.0000	7.1429	10
S0068	10.0000	5.0261	34	11.0000	6.6852	38	17.0000	10.6667	40	6.0000	5.5556	10
S0225	8.0000	4.9383	40	6.0000	3.7231	44	11.0000	7.5385	42	6.0000	5.0000	10
S0155	11.0000	7.3673	38	11.0000	7.4118	42	8.0000	4.6421	42	5.0000	3.5714	10
SW122	9.0000	5.7760	38	11.0000	7.2727	40	7.0000	4.6512	40	5.0000	3.8462	10
S0355	8.0000	3.1934	34	9.0000	3.9454	38	11.0000	8.8767	36	7.0000	4.6667	14
S0386	9.0000	6.7368	32	8.0000	5.0490	38	8.0000	3.1075	34	5.0000	3.8462	10
SW911	6.0000	4.2056	30	10.0000	7.9024	36	10.0000	6.8211	36	7.0000	6.0000	12
SW24	7.0000	4.9689	40	11.0000	7.1707	42	8.0000	6.2913	36	5.0000	4.1667	10
Mean	9.1667	5.9662	36	9.9167	6.1379	40	10.7500	7.0940	39	6.5000	5.1893	12
St. Dev	2.0817	1.8281		1.9752	1.4625		3.5194	2.7418		1.7321	1.5487	

Observed number of alleles and Effective number of alleles were calculated according to Kimura and Crow (1964).

Table 3.11 Expected Heterozygosity, Observed Heterozgosity and Nei's expected heterozygosity in four pig populations

Locus	Pop 1 (Chinese Qianbei black pigs)					Pop 2 (South Thai pigs)				
	Exp_Het	Obs_Het	Nei_Het	Ave_Het	Smpl Size	Exp_Het	Obs_Het	Nei_Het	Ave_Het	Smpl Size
S0227	0.8628	0.8000	0.8413	0.8188	40	0.7987	0.6500	0.7788	0.8188	40
S0090	0.8414	0.7333	0.8133	0.8067	30	0.8692	0.5500	0.8475	0.8067	40
S0226	0.8919	0.9474	0.8684	0.8665	38	0.8541	0.7273	0.8347	0.8665	44
S0005	0.9270	0.8889	0.9012	0.8864	36	0.8876	0.5789	0.8643	0.8864	38
S0068	0.8253	0.7647	0.8010	0.8444	34	0.8734	0.8947	0.8504	0.8444	38
S0225	0.8179	0.8500	0.7975	0.7991	40	0.7484	0.6364	0.7314	0.7991	44
S0155	0.8876	0.9474	0.8643	0.8085	38	0.8862	0.6667	0.8651	0.8085	42
SW122	0.8492	0.8947	0.8269	0.8036	38	0.8846	0.9500	0.8625	0.8036	40
S0355	0.7077	0.6471	0.6869	0.7766	34	0.7667	0.6842	0.7465	0.7766	38
S0386	0.8790	0.8750	0.8516	0.7679	32	0.8236	0.5789	0.8019	0.7679	38
SW911	0.7885	0.8667	0.7622	0.8306	30	0.8984	0.9444	0.8735	0.8306	36
SW24	0.8192	0.6000	0.7988	0.8151	40	0.8815	0.7143	0.8605	0.8151	42
Mean	0.8415	0.8179	0.8178	0.8187	36	0.8477	0.7147	0.8264	0.8187	40
St. Dev	0.0572	0.1120	0.0562	0.0342		0.0511	0.1409	0.0495	0.0342	

Expected homozygosity and heterozygosity were computed using Levene (1949); Nei's expected heterozygosity was computed according to Nei's (1973)

Table 3.11(Continued) Expected Heterozygosity, Observed Heterozgosity and Nei's expected heterozygosity in four pig populations

Locus	Pop 3 (Northeast Thai pigs)					Pop 4 (Wild boars)				
	Exp_Het	Obs_Het	Nei_Het	Ave_Het	Smpl Size	Exp_Het	Obs_Het	Nei_Het	Ave_Het	Smpl Size
S0227	0.8908	0.7143	0.8696	0.8188	42	0.8462	0.4286	0.7857	0.8188	14
S0090	0.7932	0.5294	0.7699	0.8067	34	0.8571	0.5714	0.7959	0.8067	14
S0226	0.8966	0.9524	0.8753	0.8665	42	0.9560	1.0000	0.8878	0.8665	14
S0005	0.9436	0.6000	0.9200	0.8864	40	0.9556	0.6000	0.8600	0.8864	10
S0068	0.9295	1.0000	0.9062	0.8444	40	0.9111	0.6000	0.8200	0.8444	10
S0225	0.8885	0.7143	0.8673	0.7991	42	0.8889	0.6000	0.8000	0.7991	10
S0155	0.8037	0.6667	0.7846	0.8085	42	0.8000	0.8000	0.7200	0.8085	10
SW122	0.8051	0.7000	0.7850	0.8036	40	0.8222	0.6000	0.7400	0.8036	10
S0355	0.9127	0.4444	0.8873	0.7766	36	0.8462	1.0000	0.7857	0.7766	14
S0386	0.6988	0.5294	0.6782	0.7679	34	0.8222	0.2000	0.7400	0.7679	10
SW911	0.8778	0.8889	0.8534	0.8306	36	0.9091	1.0000	0.8333	0.8306	12
SW24	0.8651	0.8333	0.8410	0.8151	36	0.8444	0.8000	0.7600	0.8151	10
Mean	0.8588	0.7144	0.8365	0.8187	39	0.8716	0.6833	0.7940	0.8187	12
St. Dev	0.0705	0.1757	0.0695	0.0342		0.0520	0.2462	0.0505	0.0342	

Expected homozygosty and heterozygosity were computed using Levene (1949); Nei's expected heterozygosity was computed according to Nei's (1973)

Table 3.12 Hardy-Weinberg test when H1 = heterozygote deficit (Estimation of exact P-values by the Markov chain method)

Locus	Pop 1 (CQB)		Pop 2 (ST)		Pop 3 (NT)		Pop 4 (WB)	
	P-value	S. E	P-value	S. E	P-value	S. E	P-value	S. E
S0227	0.3219	0.0153	0.0006*	0.0003	0.0215	0.0040	0.0113	0.0015
S0090	0.2794	0.0077	0.0018 *	0.0005	0.0006*	0.0003	0.0131	0.0018
S0226	0.9010	0.0081	0.0025*	0.0015	0.7271	0.0197	1.0000	0.0000
S0005	0.3530	0.0182	0.0000*	0.0000	0.0000*	0.0000	0.0255	0.0052
S0068	0.3478	0.0151	0.6121	0.0158	1.0000	0.0000	0.0713	0.0044
S0225	0.8416	0.0072	0.1896	0.0057	0.0419	0.0054	0.0909	0.0050
S0155	0.9048	0.0081	0.0124	0.0028	0.0038*	0.0007	0.3624	0.0062
SW122	0.3895	0.0119	0.7827	0.0117	0.0161	0.0019	0.1580	0.0048
S0355	1.0000	0.0000	0.9296	0.0067	0.0000*	0.0000	1.0000	0.0000
S0386	0.1568	0.0095	0.0664	0.0050	0.0149	0.0023	0.0029*	0.0006
SW911	0.8849	0.0041	0.6881	0.0127	0.5240	0.0134	1.0000	0.0000
SW24	0.0199	0.0021	0.0000 *	0.0000	0.0926	0.0051	0.5691	0.0067

Markov chain parameters for all tests: Dememorization=1000; Batches= 300; Iterations per batch =1000. bold value mark with * are heterozygote deficit significantly (Bonferroni correction P<0.05/12= 0.0041).

Table 3.12(Continued) HWE test when H1 = heterozygote excess (Estimation of exact P-values by the Markov chain method)

Locus	Pop 1 (CQB)		Pop 2 (ST)		Pop 3 (NT)		Pop 4 (WB)	
	P-value	S. E	P-value	S. E	P-value	S. E	P-value	S. E
S0227	0.7768	0.0136	0.9996	0.0003	0.9819	0.0033	0.9968	0.0007
S0090	0.7997	0.0067	0.9987	0.0005	0.9997	0.0002	0.9950	0.0011
S0226	0.2424	0.0125	0.9975	0.0011	0.2619	0.0185	0.7338	0.0185
S0005	0.7673	0.0160	1.0000	0.0000	1.0000	0.0000	1.0000	0.0000
S0068	0.7681	0.0125	0.5115	0.0166	0.2181	0.0189	0.9913	0.0013
S0225	0.2243	0.0083	0.8083	0.0061	0.9494	0.0063	0.9872	0.0016
S0155	0.2764	0.0139	0.9874	0.0029	0.9957	0.0011	0.7212	0.006
SW122	0.6339	0.0116	0.2659	0.0142	0.9782	0.0024	0.9740	0.0020
S0355	0.0184	0.0026	0.1558	0.0094	1.0000	0.0000	0.2597	0.0094
S0386	0.8734	0.007	0.9356	0.0049	0.9925	0.0014	1.0000	0.0000
SW911	0.2193	0.0060	0.4274	0.0131	0.5318	0.0152	0.5726	0.0109
SW24	0.9818	0.0018	1.0000	0.0000	0.8949	0.0058	0.7806	0.0053

Markov chain parameters for all tests: Dememorization=1000; Batches= 300; Iterations per batch =1000. heterozygote excess

(Bonferroni correction P>0.05/12= 0.0041)

Table 3.13 Probability values for Fisher's combined test of genic differentiation at 12 microsatllite loci (a) using uncorrected data and (b) corrected data for the presence of null alleles (uncorrected data).

P	S0227	S0090	S0226	S0005	S0068	S0225	S0155	SW122	S0355	S0386	SW911	SW24
CQB&ST	0.00000	0.00000	0.02686	0.00088	0.00000	0.00000	0.00000	0.00092	0.00027	0.00618	0.00000	0.00000
CQB&NT	0.00000	0.06246	0.00653	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
CQB&WB	0.00001	0.00006	0.07730	0.00213	0.00072	0.07135	0.00183	0.00252	0.00000	0.13867	0.00000	0.00000
ST&NT	0.00051	0.00000	0.00087	0.00434	0.00000	0.00052	0.00654	0.03823	0.00000	0.00000	0.22402	0.00000
ST&WB	0.0029	0.00011	0.29009	0.01246	0.00039	0.00004	0.02530	0.97659	0.00000	0.00013	0.81146	0.00266
NT&WB	0.07703	0.00005	0.74658	0.14352	0.00157	0.08121	0.05924	0.09386	0.00000	0.01908	0.72175	0.00056

P = Population; CQB = Chinese Qianbei Black pigs; NT = Northeast Thai pigs; ST =South Thai pigs; WB = Wild Boars.

Table 3.13(Continued) Probability values for Fisher's combined test of genic differentiation at 12 microsatllite loci (a) using uncorrected data and (b) corrected data for the presence of null alleles (uncorrected data).

P	S0227	S0090	S0226	S0005	S0068	S0225	S0155	SW122	S0355	S0386	SW911	SW24
CQB&ST	0.00000	0.00000	0.02461	0.00107	0.00000	0.00000	0.00000	0.00052	0.00049	0.00366	0.00000	0.00000
CQB&NT	0.00000	0.06098	0.00609	0.00033	0.00015	0.00000	0.00000	0.00000	0.00000	0.00005	0.00000	0.00000
CQB&WB	0.00019	0.00011	0.07967	0.00191	0.00122	0.07744	0.00192	0.00364	0.00000	0.14122	0.00000	0.00000
ST&NT	0.00017	0.00000	0.00022	0.00410	0.00000	0.00039	0.00604	0.02943	0.00000	0.00000	0.22686	0.00000
ST&WB	0.00426	0.00004	0.29398	0.01521	0.00038	0.00000	0.02604	0.97819	0.00000	0.00003	0.81528	0.00290
NT&WB	0.08068	0.00000	0.74735	0.12954	0.00227	0.07143	0.06628	0.09418	0.00000	0.01773	0.72567	0.00072

P = Population; CQB = Chinese Qianbei Black pigs; NT = Northeast Thai pigs; ST =South Thai pigs; WB = Wild Boars.

Table 3.14 Nei's standard genetic distance (below diagonal) and Nei's unbiased genetic distance (above diagonal) among four pig populations.

	CQB	**ST**	**NT**	**WB**
CQB	0.0000	0.9459	0.9801	1.0682
ST	1.0129	0.0000	0.7124	0.7586
NT	1.0499	0.7791	0.0000	0.5104
WB	1.2020	0.8894	0.6440	0.0000

Notes: CQB = Chinese Qianbei Black pigs; ST = South Thai pigs;
NT = Northeast Thai pigs; WB = Wild boars

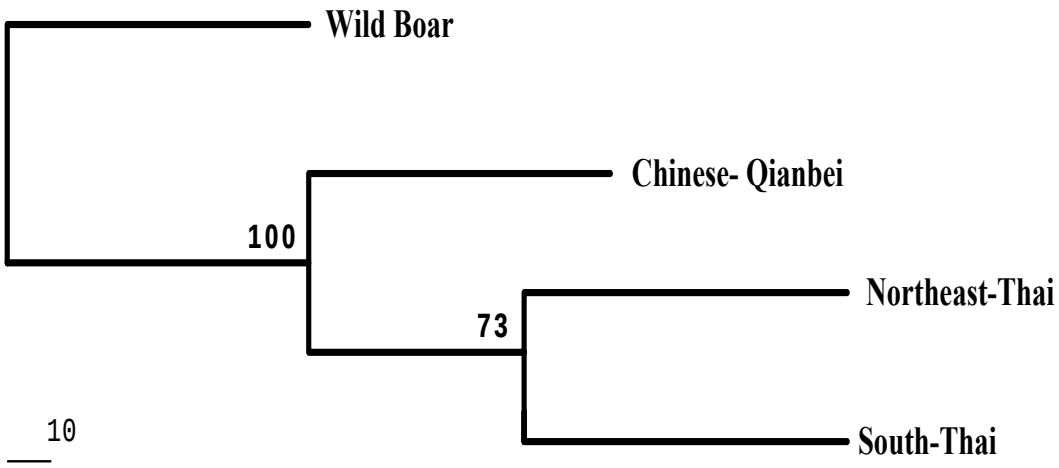

Figure 3.18 UPGMA tree showing the genetic relationships among four pig populations from Nei's standard distance (Nei, 1972) based on data of 12 microsatellite markers. The numbers at the nodes are percentage bootstrap values from 1,000 replications of re-sampled loci.

3.5 Discussion

3.5.1 About DNA source and DNA template concentration

A large number of reports on extracting DNA for genetic analysis have been found (Baumung et al., 2004; Linda et al., 1999). Most of papers suggested that DNA extracted from hair roots was enough for PCR reactions based on mtDNA, but PCR amplification was slightly confined because of small amount of genomic DNA and presence of inhabitants (Goldberg et al., 1997). Different DNA template concentrations were applied to various genetic analyses, The most preferable amount was 10 ～50 ng/μL. In our experiment, 2.5 ng/μL and 5 ng/μL of DNA template concentrations may obtain PCR products which can be employed to run PAGE. Microsatellite primers S0225 and S0227 were taken from the recommendation loci by FAO/ISIG; these two primers could acquire ideal PCR products in most of genetic diversity studies. It has been demonstrated that our results were not influenced by selection of microsatellite primers.

Some reports regarding the correlation between number of hair roots and DNA concentrations have been found. Reginaldo et al. (2000) compared the amplification effects using different DNA templates taken from 1, 2, and 3 hair roots respectively, the results showed that DNA amount from only 1 hair root was enough for PCR amplification for Halothane gene; no significant difference could be observed between DNA template from 1 hair root and from 2, 3 hair roots, respectively. However, this study applied NaOH method for DNA extraction. In present experiment, the Wizard Genomic DNA Purification Kit was used to extract DNA, and DNA extracted from 100 hair roots and 200 hair roots were used to compare the effects for PCR reaction, 0.7 % agarose gel electrophoresis and OD_{260} measuring indicated that

obvious relationship between number of hair roots and DNA concentrations could not be found. One possible reason is due to purification degree of DNA. Also, the presence of protein may cause an increase of OD value.

Polyacrylamide gel electrophoresis is an important tool in animal genetic diversity studies because of its higher degree of sensitivity and distinguishing rate, even 1～2 base pairs can be identified in PAGE (Reiner et al., 1997). Accordingly, higher requirements for DNA quality are needed in PAGE. Poor DNA quality may produce fuzzy bands or no band can be viewed. If the amount of DNA is not enough, lower density bands will occur at the bottom of electrophoresis plate. In this experiment, of all 21 hair roots samples, although some of PCR products could not obtain much clear bands in 2% agarose gel, PAGE results indicated very clear bands, suggesting that DNA quality and quantity were able to meet the requirement for microsatellite PCR and 6% polyacrylamide gel electrophoresis.

3.5.2 HWE TEST

In present study, all populations were in Hardy-Winberg equilibrium, but for 7 of 12 loci were significantly deviated from HWE ($P< 0.05$). The disequilibrium might be cause by genotyping error, null alleles, non-random sampling or inbreeding. Hardy-Winberg test has shown no heterozygote excess in all loci in all populations (Table 3.12, $P< 0.05$). Deficiency of heterozygotes was probably caused by the *Walhund effect,* which has been proposed in other domestic pigs such as Mexican hairless pig population (Lemus-Flores et al., 2001).

3.5.3 Genetic variations

Although, in the past, there was not sufficient data for recording genetic variations, present study showed observed mean number of alleles and effective number of alleles had higher values in NT (10.75; 7.09) and ST (9.92; 6.3) populations than that of CQB (9.17; 5.97) and WB (6.5; 5.19) populations, also higher than the European pig populations (Laval et al., 2000) and some Chinese pig populations (Fang et al., 2005; Li et al., 2004; Fan et al., 2002). The mean numbers of allele per locus in NT and ST populations were higher than previous study (Chaiwatanasin et al., 2002). Results suggested that no population bottleneck occurred in Thai indigenous pig populations in the past decades. Conversely, a relatively low *Ne* value in WB population (5.19) reflected a smaller WB population, which might caused by bottleneck effect. During the process of sampling, we found some crossbreds with wild boars and Chinese Meishan pigs. It was able to lead to reduction of number of wild boars.

As to the values of heterozygosity, we focused on H_E because it is considered a better estimator of the genetic variability present in a population (Nei and Kumar, 2000). As shown in Table 3.9, Wild boar, Northeast Thai pigs, South Thai pigs and Chinese Qianbei Black pigs exhibited a high degree of genetic diversity compared with European pig populations (Laval et al., 2000) and some Chinese pig populations mentioned above, H_E values of Thai indigenous pig were higher than that of Korean native pig breeds (Kim et al., 2002 and Kim et al., 2005), also slightly higher than that of native pigs of India (Behl et al., 2006). This H_E value is a little bit higher than previous study (H_E = 0.77) reported by Chaiwatanasin et al. (2002), these results indicated there exist a relatively large indigenous pig population in Thailand. Another possible reason is due to apply different microsatellite markers. The high

heterozygosity levels present in Thai indigenous pigs may be the result of low rate of selection pressure owing to the lack of improvement programs. In the past decades, although the Thai government has been recognized as an important promoter of genetic resource, there have not been preservation farms for conservation strategy. All samples were taken from individual farmer's backyards; there were few crosses between indigenous pigs and commercial breeds. In addition, high genetic diversity in Thai native pig can also be attributed to its breeding history and traditional customs in raising pigs, including good pork quality, low-consuming ration feeding way, and higher pork price for providing market, and so forth.

3.5.4 Phylogeny relationship

Two factors are considered when constructing phylogenetic trees in our study; firstly, Neighbor-Joining method is preferable because it is used to be less affected by the presence of admixture occurring among populations in covering the correct topology compared with the unweighted pair-group method of averages (UPGMA). Second, according to the survey for global breed diversity studies (Baumung. R. et al., 2004), the most favored measure is Nei's standard genetic distance Ds (Nei, 1972). This measure was used in 74% of all projects; they especially suggested Nei's standard genetic distance to be more useful to with respect to reconstruction the topology than other genetic distances such as Cavali Sforza and Edwards' chord distance (Cavali-Sforza and Edwards, 1967) and Reynolds's distance (Reynolds et al., 1983). Therefore, we used Nei's standard genetic distance for construction of Dendrograms.

As shown in Figure 3.18, two Thai indigenous pig populations ST and NT

were classified as the same branch(73% bootstrap support), and then were clustered into the same branch with Chinese Qiabei Black pigs (CQB) with a 100 % of the bootstrap value. But WB population was classified as another lineage. The result suggested that Chinese Qianbei Black pigs had a closer genetic relationship with NT and ST population than that with WB population. Moreover, the geological distances between Chinese southern region and northeast Thailand region is not far. We earlier mentioned in former part that there are some marvelous similarities with respect to body size, morphology, and coat color even in productive performances between Thai indigenous pigs and Chinese Qianbei Black pigs. Chaiwatanasin et al. (2002) reported that North Thai pigs had a close genetic distance (0.55), geological distance, and similar genetic variations with Northeast Thai pigs. The current result points to a common ancestor between Thai native pigs and Chinese Qianbei Black pigs. Chinese breeds were classified into six types according to their geographic origin, distribution, body conformation, and coat color (Li et al., 2004). Based on this classification, the CQB pig belongs to Type Ⅲ (Southwest China), although there has not been accurate documentation that can prove where Thai pigs came from. Some Asian native pig breeds such as Korean, Vietnam and Laos pigs were reported to originate from China (Kim et al., 2005; Tomowo et al., 2000); their studies suggested China is considered to be one of the major centers of origin for the domestic pigs in Asia (Tomowo et al., 2000). From these previous studies, the Thai native pig population may originate from southwest of China or south of China.

3.6 Conclusion

DNA quality and concentrations from blood and hair roots were compared; results suggested that DNA taken from 100 or 200 pig hair roots could be used for PCR reaction based on microsatellite loci, obvious differences on PCR products between blood and hair roots could not be observed. 2.5 ng/μL and 5 ng/μL of DNA template concentration could obtain clear bands. 1 ng/μL DNA concentration appeared weak band in 0.7% agarose gel electrophoresis. No significant relationship between number of hair roots and DNA concentrations could be found. PCR products based on microsatellite from all of 21 hair root samples could be used for running PAGE and scoring. It may be given a conclusion from present experiment that whole hair roots can be used as materials for pig genetic diversity studies.

In conclusion, Thai indigenous pig population had high heterozygosity and exhibited a high genetic diversity compared with some Chinese native pig breeds, European pig breeds and some Asian pigs such native pigs from India and Korean native pigs, suggesting that there still exist a large Thai indigenous pig population. An analysis of a phylogenetic tree based on 12 microsatellite markers provided a result that Chinese Qianbei Black pigs had closer genetic relationship with two Thai indigenous pig populations ST and NT, whereas WB was clustered into independent branch. Considering present results combined with previous relative researches, a conjuncture can be made that Thai native pig population may originate from southwest or south of China. These resullt can be used as genetic information and further genetic improvement of Thai indigenous pigs. However, the further studies with respect to mtDNA sequence need to be conducted to confirm its origin by

comparing indigenous pig populations from other region of Thailand, some other Chinese pig breeds and Asian pig populations.

3.7 References

APHCA (Animal Production and Health Commission for Asia and the Pacific), FAO. (2002). **The Livestock industries of Thailand.** RAP publication No. 2002/23

Baumung, B. R., Simianer, H., and Hoffmann, I. (2004). Genetic diversity studies in farm animals – a survey. **J. Anim. Breed. Genet.** 121: 361-373.

Behl, R., Sheoran, N., Behl, J., and Vijh, R. K. (2006). Genetic analysis of Ankamali pigs of India using microsatellite markers and their comparison with other domesticated Indian pig types. **J. Anim. Breed. Genet.** 123: 131–135.

Bernard and Russell, H. (2002). Research Methods in Anthropology: Qualitative and Quantitative Methods. **Walnut Creek:** AltaMira Press.

Cavalli-Sforza, L.L, and Edwards, A. W. F. (1967). Phylogenetic analysis: models and estimation procedures. **Am. J. Hum. Genet.** 19: 122–257.

Chaiwatanasin, W., and Chantsavang, S. (2002). Genetic Diversity of Native Pig in Thailand Using Microsatellite Analysis. **Kasetsart J. (Nat. Sci.)** 36: 133 – 137.

Department of Domestic Animals Development of Thailand (DDADT). (1999). Handbook for Native animals' conservation and Development.

Efron, B., Halloran, E., and Holmes, S. (1996). Bootstrap confidence levels for phylogenetic trees. **Proceedings of the National Academy of Scince** (USA) 93: 7085-7090.

Fan, B. *et al.* (2002). Genetic variation analysis within and among Chinese indigenous swine populations using microsatellite markers. **Anim. Genet.** 34: 465–466.

Fang, M. *et al.* (2005). The phylogeny of Chinese indigenous pig breeds inferred from microsatellite markers. **Anim. Genet.** 36: 7–13.

Felsenstein J., (1993). PHYLIP (Phylogeny Inference Package) Version 3.67c, **Department of Genetics, University of Washington,** Seattle.

Gilks, W., Richardson, S., and Spiegelhalter, D. (1996). Markov Chain Monte Carlo in Practice. **Chapman and Hall,** London.

Glaubitz, J. C. (2004).CONVERT: A user-friendly program to reformat diploid genotypic data for commonly used population genetic software packages. **Molecular Ecology Notes**. 4: 309-310.

Guizhou Animal and Poultry Species Committee (GAPSC). (1993). Introduction to Domestic Animals and Poultries Species of Guizhou Province. **1th ed. Guizhou Sci. and Tech.** Press, Guiyang.

ISAG/FAO, (2004). Measurement of Domestic Animal Diversity (MoDAD): Recommended Microsallite Markers. Recommendations of Joint **ISAG/FAO Standing Committee.** pp:19-24.

Kim, K. S., and Choi, C. B. (2002). Genetic structure of Korean native pig using microsatellite markers. **Kor. J. Genet.** 24: 1–7.

Kim, T.H. *et al.* (2005). Genetic structure of pig breeds from Korea and China using microsatellite loci analysis. **J. Anim. Sci.** 83: 2255–2263.

Kimura, M., and Crow, J. F. (1964). The number of alleles that can be maintained in a finite population. **Genetics.** 49: 725–738.

Laval, G., *et al.* (2000). Genetic diversity of eleven European pig breeds. **Genet. Sel. Evol.** 32: 187–203.

Leneve, H. (1949). On a matching problem arising in genetics. **Ann. Math. Stat.** 20: 91-94.

Lemus-Flores, C., Ulloa-Arvizu, R., Ramos-Kuri, M., Estrada, F. J., and Alonso, R. A. (2001). Genetic analysis of Mexican hairless pig populations. **J. Anim. Sci.** 79: 3021–3026.

Li, S. J. *et al.* (2004). Genetic diversity analyses of 10 indigenous Chinese pig populations based on 20 microsatellites. **J. Anim. Sci.** 82: 368–374.

Martin-Burriel, I., Garcia-Muro, E., and Zaragoza, P. (1999). Genetic diversity analysis of six Spanish native cattle breeds using microsatellites. **Anim. Genet.** 30: 177–182.

Minch E., MICROSAT Version 1.5b (Macintosh). (1998). **University of Stanford, Stanford.**

Nei, M. (1972). Genetic distance between populations. **Am Nat.** 106: 283-292.

Nei, M. 1973. Analysis of gene diversity in subdivided populations. **Proc Natl Acad Sci.** USA 70: 3321-3323.

Nei, M. (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. **Genetics.** 89: 583-590.

Nei, M., and Kumar, S. (2000). Molecular Evolution and Phylogenetics. **Oxford University Press**, New York, NY.

Pongchan Na-Lampang. A study on biodiversity of native pig in the Northeast. A complete report. **Suranaree University of Technology**. P. 2002.

Pongchan Na-Lampang. A study on factors affecting the conservation of genetic resource of Thai pigs in Northeast Thailand. A complete report: **Suranaree University of Technology.** P. 2002.

Raymond, M., and F. Rousset. (1995). GENEPOP (Version 1.2): population genetics software for exact tests and ecumenicism. **J. Heredity.** 86:248-249.

Reynolds, J., Weir, B. S., and Cockerham, C.C. (1983). Estimation of the coancestry coefficient basis for a short-term genetic distance. **Genetics.** 105: 767–779.

SanCristobal, M. *et al.* (2006). Genetic diversity in European pigs utilizing amplified fragment length polymorphism markers. **Animal Genetics**. 37: 232–238.

Shipley, P., (2003). Micro-Checker, Version 2.21. **University of Hull**, Hull, UK. Available from http://www/microcheck.hull.ac.uk/.

Tanaka, Kazue. (1974). Morphological and serological studies on the native pigs in Thailand. **Report of the Society for Research on Native Livestock.** 6: 181-183.

Tomowo, O. *et al.* (2000). Molecular Phylogenetic Analysis of the Wild Boars and Native Domestic Pigs in Laos. **Pep, Soc. Res. Native Livestock**. 18: 149-158.

Yeh, F. C., Yang, R. C., Boyle, T. B. J., Ye, Z. H., and Mao, J. X. (1997). POPGENE. The User-friendly Shareware for Population Genetic Analysis. Molecular Biology and Biotechnology Centre, **University of Alberta**, Can.

Zhang, G. X.*et al.* (2003). Genetic Diversity of Microsatellite Loci in Fifty-six Chinese Native Pig Breeds. **Acta Genetica Sinica.** 30(3): 225-233.

CHAPTER IV

ANALYSIS OF THE PHYLOGENETIC RELATIONSHIPS AMONG SOUTH THAI PIGS AND THAI WILD BOARS AND CHINESE QIANBEI BLACK PIGS IN TERMS OF SEQUENCE POLYMORPHISM OF *mt*DNA *Cyt* b GENE

4.1 Abstract

To study the phylognetic relationships of indigenous Thai pigs, *Cyt* b gene fragment from 17 samples based on three pig breeds were checked, 7 of which was came from southern region of Thailand, 8 of which came from Chinese Qianbei Black pigs, 2 of which were derived from Wild Boars in Thailand. DNA extraction and PCR amplification were performed according to the Co. QIAGEN's protocol. PCR products were purified and sequenced. A total of the five haplotpyes and eight polymorphic nucleotide sites were detected. Only one haplotype (HCS) was found in South Thai pigs (ST population) from seven ST individuals, and shared the haplotype with the other Chinese Qianbei black pigs (CQB population), the average haplotype frequency was relatively low (29.4%). (A+T) content (57.2-57.3%) in all haplotypes were more than (G+C) content (42.7%-42.8%), the restrictive enzyme cutting positions were also determined by using a computer software GENETYX-WIN (version 3.1). The result showed that three restriction enzymes (Stu *I*, Tai *I*, and Taq *I*) had identical cutting positions in Haplotype HC1, HC2, HWB1 and HWB2 except

HCS; Restriction enzyme *Mbo Π* had identical cutting positions in haplotypes HC1, HC2, HCS, and HWB2 except for HWB1. Neighbor-Joining method was applied to construct phylogenetic tree and result indicated that ST population had much closer genetic relationship with CQB rather than WB population. This result is consistent with that study on phylogenetic relationship based on microsatellite markers stated in Chapter Ш. An conjecture could be made that Thai indigenous pigs maybe originate from south or southwest of China.

4.2 Introduction

Pork has become the second most important meat in Thai consumption, but intensive pig production started in 1960 (FAO, 2002). As to Thai indigenous pigs, according to Takana (1981), there are three types of indigenous Thailand pigs. Hailum, primarily distributed in the south and the central areas of Thailand; Murad, mostly distributed in the north, the northeast and the south in Thailand; Mukuai, mainly found in the north and the central areas of Thailand. Together these populations maybe represent, to some extend, phenotypic diversity in Thai indigenous pigs. Some populations such as South Thai pigs are considered to have a small population size, and are under increasing pressure from the introgression of modern commercial breeds. Another one small pig population, wild boars living in Thailand, we could not understand their phylogenetic history. This makes investigations of both population structure and genetic diversity increasingly important.

Mitochondrial DNA has been widely used to perform phylogenetic studies in different animal species. In pigs, genetic variability at the cytochrome b (*Cyt* b) gene and the D-loop region has been used as a tool to dissect the genetic relationships

between different breeds and populations (Alex *et al.*, 2004).

Most of previous studies were to determine the phylogenetic relationships among varieties of pig populations by using direct sequencing of the main non-coding mtDNA region (D-loop) and *Cyt b* gene. Randi *et al*. (1996) used *Cyt* b polymorphism for evolutionary analysis of the suiformes and also to determine relationships among some *Sus scrofa* populations. Alves *et al*. (2003) used nucleotide sequences of *Cyt* b gene and control region to determine the phylogenetic relationships among ancient and current varieties of Iberian pigs. Alex *et al.* (2004) analysed four SNP at the *Cyt* b gene to infer the Asian or European origins of several European standard and local pig breeds. Giuffra *et al*. (2000) studied the genetic relationship based on mtDNA between domestic pigs and wild boars; studies on molecular phylogenetic relationship based on mtDNA between Chinese native pig breeds and European breeds have been reported (Jiang *et al*., 2001; Yang *et al.,* 2003). Molecular phylogenetic studies regarding other Asian pig breeds and wild boars living in Laos、Japan、and Vietnam have been performed (Watanobe *et al.*, 1999; Hongo *et al.*, 2002). These authors presented clear evidences of the independent domestication events of European and Asian subspecies of wild boar.

However, genetic variability, phylogenetic study of indigenous Thai pig populations and wild boars living in Thailand based on mtDNA, remain largely unknown. Historic changes and migration on Thai indigenous pigs are poor documented. Moreover, the phylogenetic analyses in previous studies mentioned above did not involve outgroup comparison, which was necessary to assess inner group relationships among individuals from wild boars and domestic pigs. The purpose of this research was to assess genetic diversity and phylogenetic relationship

based on mtDNA *Cyt* b gene among several indigenous pig populations living in Thailand、also involving Chinese Qianbei pig breed.

4.3 Materials and Methods

4.3.1 Selection of samples

Hair roots samples as described in Experiment III were selected partly to conduct mitochondrial DNA analysis, the same populations except NT pigs were used, but the sizes of samples were relatively smaller because of DNA quality and PCR effects. Finally, 7 South Thai pig samples from Nakhon Si Thammarat province, south of Thailand. 8 CQB hair roots samples from Chinese Qianbei balck pigs and 2 Wild Boar samples from Sakon Nakhon, Nakhon Phnom province in Northeast of Thailand were employed. A total of 17 samples were also be used for analysis on molecular phylogenetic relationship.

4.3.2 Genomic DNA extraction

Wizard Genomic DNA Purification Kit were used for DNA extraction as described in Chapter III.

4.3.3 Amplification of the *Cyt* b gene

A total of 1046bp (14097-15243) of *Cyt* b gene was amplified using a set of oligonucleotide primer, and synthesized by Bioiogenomed CO., Ltd., Thailand.

The design of primer was refered to Mit L1 and MitH2 (Watanabe et al., 1999),

MitL1 5’-ATCGTTGTCATTCAACTACA-3’

MitH2 5’-CTCCTTCTCTGGTTTACAAG-3’

The primer sequences of cytochrome b gene in this experiment were as follows,

5’CAAGACGTTGTAAAACGACGAATTCATCGTTGTCATTCAACTACA-3’ (forward)

5’GGATAACAATTTCACACAGGGAATTCCTCCTTCTCTGGTTTACAAG-3’ (reverse)

PCR were performed in 10 μL of reaction mixture containing 10 ng/μL of template DNA, 10×buffer, 2.5 mM dNTP mixture, 10 pmol primer and 0.25 unit of Ex *Taq* DNA polymerase. The amplification was performed in iCycler PCR system (BIO-RAD, USA) with an initial cycle at 95°C for 30 sec followed by 35 cycles at 95°C for 45 sec and 55°C for 30 sec and followed by 72°C for 1 min. 72°C extension for 7 min. PCR products were checked using 0.7% agarose gel electrophoreses. Then, 40 μL of reaction volume of PCR was performed using the same reaction conditions.

4.3.4 DNA purification from agarose gel

The total 50 μL of PCR products were run 0.7% agarose gel electrophoreses, the gel were be cut and purified with QIA quick PCR Purification kit from Gel according to the Co. QIAGEN’s protocol. The amplified DNA fragments were determined directed by the dye terminator methods (Takumi *et al*., 1997) by Macrogen Co. in South Korea. The purified PCR products were sequenced by mailing to Macrogen Co. in South Korea using the relevant DNA sequencer.

4.3.5 Data analysis

GENETYX-WIN program version 3.1(Software Development Co. Ltd, Tokyo, Japan) was applied to connect the forward DNA fragment and reverse DNA

fragment, the final length was 1046bp, the majority of *Cyt* b gene sequences (91.7%) were aligned using GENETYX-WIN, of haplotypes were determined using CLUSTAL X program version 1.8 (Higgins *et al.,* 1988). Levels of genetic variability were estimated as the number of polymorphic sites and haplotype diversity (*h*) (Nei, 1987) and nucleotide diversity (π) (Tajima, 1981) using MEGA 4.0 (Kumer *et al*., 2004). After the sequences of all haplotypes were obtained, the restriction sites for five haplotypes were determined by using GENETYX-WIN program.

Pairwise genetic distances among mtDNA haplotypes were estimated across all populations using Tamaru-Nei's (1993) model of evaluation using MEGA 4.0 (Kumer *et al.*, 2004). The computer package PHYLIP version 3.67 (Felsenstein, 1993) was applied to construct phylogenetic trees.

4.4 Results and Discussion

4.4.1 DNA extraction and PCR product purification

After conducting DNA extraction , DNA quality were checked with 0.7% agarose gel electrophoresis (Figure 4.1 and Figure 4.2), most of samples showed clear bands but not very intensive compared to blood samples described in Chapter III.

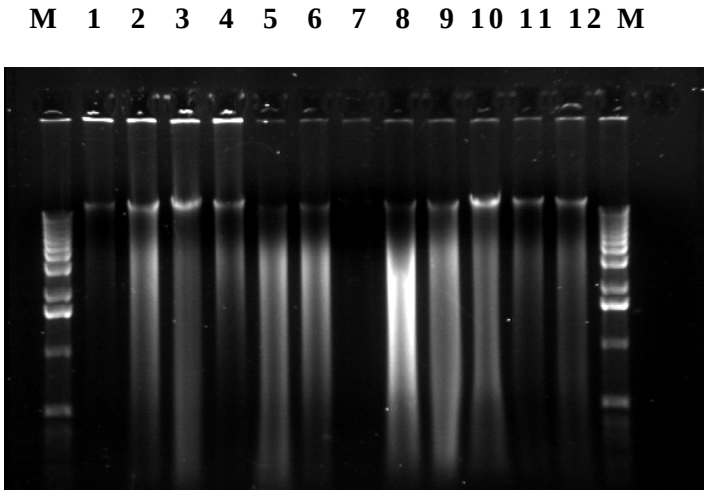

Figure 4.1 DNA extraction using 100 hairs from partly South Thai pigs

M: Lane1:S1-2; Lane3:S1-9; Lane8:S4; Lane10: S6

S: South Thai samples

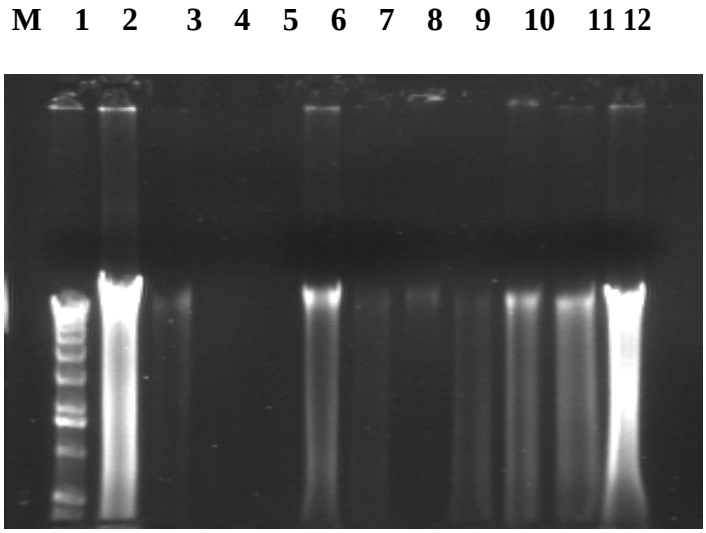

Figure 4.2 DNA extraction using 50-100 hairs form partly Chinese Qianbei black pigs

M: marker; Lane1-7: C1-7; Lane8-11: C8, C9, C10, C11

S: South Thai samples; C: Chinese Qianbei samples

After running PCR, not all the samples could get PCR products because of DNA quality and other possible reasons. Samples for good PCR products were purified and approximately 30 μL of purified PCR products could be acquired, which can be checked in 0.7% agarose gel electrophoresis as shown in Figure 4.3.

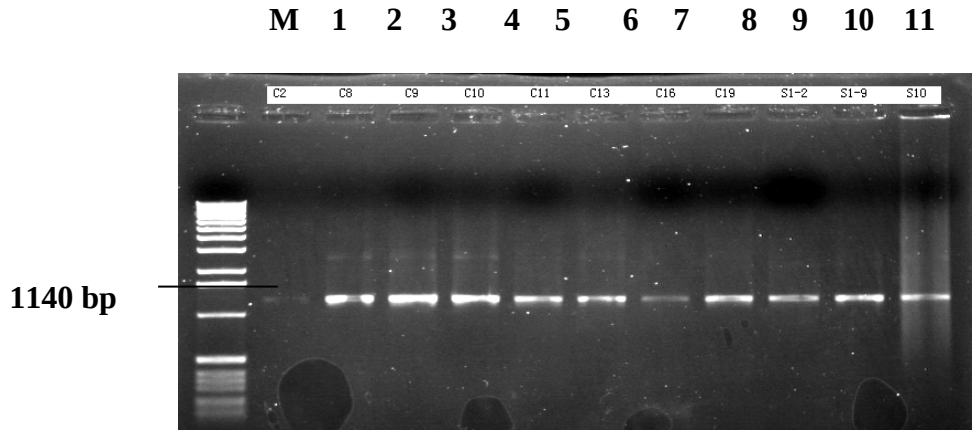

Figure 4.3 Purified mtDNA (*Cyt b* gene) from Chinese Qianbei black pigs and South Thai pigs

Lane1 –lane11: C2, C8, C9, C10, C11, C13, C16, C19, S1-2, S1-9, S10

C: Chinese Qianbei pig samples;

S: South Thai pig samples

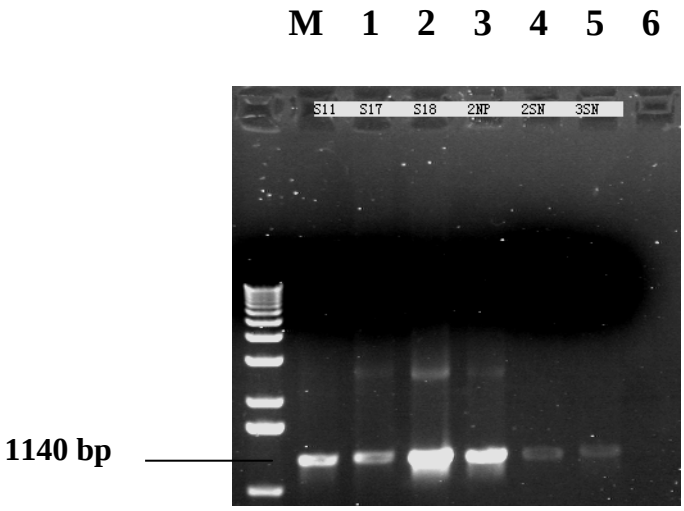

Figure 4.3 (Continued.) Purified mtDNA (*Cyt* b gene) from Chinese Qianbei Black pigs and South Thai pigs

S11, S18, S16: samples from South Thai pig; 2NP, 2SN, 3SN: Samples from wild boars

From Figure 4.3, we found that Chinese pig C2, wild boar 2SN and 2NP could not show intensive bands.

4.4.2 Number of haplotyes and nucleotide composition

1046bp of *Cyt* b gene fragment (91.7% of whole *Cyt* b gene) in all of the five haplotpyes were listed in Figure 4.4. Only five different haplotypes and 8 polymorphic nucleotide sites were found among 17 sequences of three pig populations.

Table 4.1 showed the number of haplotypes shared among several pig populations. The results could be shown from the total of 17 samples, only one haplotype (HCS) was found in South Thai pigs (ST population), and shared the

Table 4.1 Number of haplotypes shared among pig populations

Haplotype	**Number**	**Distribution**	**(A+T)%**	**(G+C)%**
HC1	2	CQB: C9, C13	57.2	42.8
HC2	1	CQB: C11	57.2	42.8
HCS	12	ST: S1-2, S1-9, S4, S6, S11, S14, S18;	57.3	42.7
		CQB: C8,C10,C15,C16, C19	57.3	42.7
HWB1	1	WB: 2NP	57.2	42.8
HWB2	1	WB; 2SN	57.2	42.8

HC1= haplotype1 for CQB pig; HC2 = haplotype2 for CQB pig; HCS = Share haplotype for CQB pigs and ST pigs; HWB1 = haplotype 1 for WB; HWB2 = haplotype 2 for WB.

haplotype with the other Chinese CQB, in other words, the *Cyt* b gene fragments from seven ST individuals were completely identical with that of five CQB individuals. Two wild boars produced two sorts of haplotypes (HWB1 and HWB2) respectively, the other haplotypes (HC1; HC2,) were occurred within CQB population.

The average frequency of haplotype for three populations was 29.4%, which was relatively low compared to other native pig breeds. In particular, ST pig population produced only one haplotype from seven samples, the frequency of haplotype was 14.3%, for CQB population, three haplotypes were detected from eight samples, the haplotype proportion was 37.5%, whereas haplotype proportion for WB population was 50%. The possible reason for low haplotype proportion may be caused

by limited sample number and sampling sites, especially for ST pigs, seven samples only came from one province in Thailand, the relative small selective areas for sampling may led to lower genetic diversity. We once described in chapter Ⅲ only seven WB samples could be collected, unfortunately, four of them could obtain PCR products, and only two WB samples could get sequencing results. Another reason for low haplotype in this research is probably due to selection, it is worth mentioning that CQB samples came from the conservation pig farm, in order to maintain the consistency of these native pigs, to some extent, selective mating may be carried out in this farm.

(A+T) content (57.2-57.3%) in all haplotypes were more than (G+C) content (42.7%-42.8%), and average contents for (A+T) and (G+C) were similar.

4.4.3 Nucleotide variable site and sequence polymorphism

Table 4.2 indicates all the variable positions in *Cyt* b gene of mtDNA in five haplotypes, only eight polymorphic sites were detected, three of them showed transition substitutions, and the other five transversion substitutions. The value of nucleotide diversity (π) was 0.00325, indicating that nucleotide diversity was relatively low. Haplotpye diversity was 29.4%, it was also considerable lower.

Table 4.2 Variable positions in *Cyt* b gene of mtDNA.

Haplotype	Variable position								Number of animal
	480	**556**	**569**	**579**	**582**	**586**	**826**	**988**	
HC1	C	T	C	A	A	A	A	G	2
HCS	C	.	.	.	.	.	A	A	12
HWB1	T	.	.	.	.	.	G	.	1
HWB2	C	.	.	.	.	.	G	.	1
HC2	C	A	A	T	C	C	A	A	1

4.4.4 Determination of restrictive cutting positions

Approximately 170 sorts of restriction enzymes listed in computer program GENETYX-WIN were used to search restrictive cutting positions for five haplotypes, experiment was performed using computer program, these restriction enzymes were derived from seven companies including NEB97, Npgene, Promega, ResFile, Stratagn, Takara, and ToyoB97. After running the computer program, recognized positions ranged from 1～13, and could be found among five haplotypes by means of restriction enzymes. But four restriction enzymes were special, the recognized positions could not be found in all five haplotyoes. The identical restrictive cutting positions could be detected in four haplotypes (HC1, HC2, HWB1, and HWB2) after using restriction enzymes (*Stu I, Tai I, and Taq I*), no recognized positions could be detected in haplotyoe HCS (Table 4.3), while the same restrictive cutting positions could be found in four haplotypes (HC1, HC2, HCS, and HWB2) when using enzyme *Mbo Π*, no recognized positions existed in haplotyoe HWB1.

Table 4.3 Cutting positions of restriction enzymes in 1046bp of mtDNA *Cyt* b gene fragment

Haplotype	Restriction Enzyme	Recognised sequence	Recognized positions	Note
HC1, HC2, HWB1, HWB2	*Stu I*	AGGCCT	84, 243	No recognized positions in HCS
	Tai I	ACGT	158, 233, 335, 786	
	Tag I	TCGA	153, 452, 894	
HC1, HC2, HCS, HMB2	*Mbo II*	GAAGA/TCTTC	476	No recognized positions in HWB1

4.4.5 Phylogenetic tree based on the difference of haplotypes

The pair wise genetic distance using Timura 2-parameter method based on five haplotypes were computed using MEGA 4.0, results indicated that the smallest values of genetic distances could observed between HWB1 and HWB2, HCS and HWB2, and HC1 and HCS (Table 4.4).

Table 4.4 Pairwise genetic distance based on five haplotypes using Timura 2-parameter distances.

	HC1	**HCS**	**HWB1**	**HWB2**	**HC2**
HC1					
HCS	0.0010				
HWB1	0.0029	0.0019			
HWB2	0.0019	0.0010	0.0010		
HC2	0.0058	0.0048	0.0067	0.0058	

Phylogenetic tree was constructed using 1046bp of sequences *Cyt* b gene based on five different haplotypes from 17 pig hair roots samples (Figure 4.5). HC2 and HCS were classified as one clade with 57% of bootstrap support, and then were subclustered with HC2 (66%), and then subclustered with Thai wild boar haplotype HWB2. This suggests that Chinese Qianbei Black pigs had much close genetic relationship, whereas Haplotype HWB1 was grouped into another lineage. UPGMA method was also used to construct phylogenetic tree to compare the topology based on the same information as the Neighbor-joining method, the same results were detected. Both of these two results are consistent with our study on phylogeny among NT, ST, CQB, and WB pig populations using microsatellite DNA described in chapter Ⅲ.

There are more than 200 domesticated pig breeds in the world, about 30% of these are from China and another 33% originate from Europe according to the domestic animal diversity information system of the Food and Agricultural Organization (http://dad.fao.org/en/home. htm). Zhang (1986) described 48 Chinese indigenous pig breeds, which were classified into six types according to their

geographic origin, distribution, body conformation and color. Based on this classification, Chinese Qianbei Black pig breed was classified as South west type (Type V), whereas Hailan pig was put into the South China type (Type IV). As we described in Chapter Ⅲ, there was a marvelous similarity in body size and conformation among Hailum pig and Mukuai pig in Thailand and Qianbei Black pig in Guizhou (CQB). Maybe these pig breeds came from a common ancestor. That is another purpose that we used Chinese Qianbei black pig breed for experimental material. Considering geographic position, there are a closer distance between Thailand and Chinese southern and southwestern regions. Although historic migration and changes in Thai pigs still remains unknown, it is quite possible that introgression occurred among pig breeds located in these regions. We also studied the phylogenetic relationships using microsatellite markers among ST, NT, CQB, and WB pig population as stated in Chapter Ⅲ, the result showed that ST population was clustered as same group with NT population, and then classified into subcluster with CQB population, wild boars was classified as a independent group. Similar result could be detected in study of phylogenetic relationship based on *Cyt* b gene as mentioned above. An important result in this study was the 1046 bp sequences fragments of *Cyt* b gene from seven ST individuals were completely identical with that of five CQB individuals.

Summarizing the facts obtained in theses studies, it may be made an conjecture that Thai indigenous pigs were introduced from south or southwest of China.

```
HWB1          1:GACCTCCCAGCCCCCTCAAACATCTCATCATGATGAAACTTCGGTTCCCTCTTAGGCATC 60
HWB2          1:GACCTCCCAGCCCCCTCAAACATCTCATCATGATGAAACTTCGGTTCCCTCTTAGGCATC 60
HCS           1:GACCTCCCAGCCCCCTCAAACATCTCATCATGATGAAACTTCGGTTCCCTCTTAGGCATC 60
HC2           1:GACCTCCCAGCCCCCTCAAACATCTCATCATGATGAAACTTCGGTTCCCTCTTAGGCATC 60
HC1           1:GACCTCCCAGCCCCCTCAAACATCTCATCATGATGAAACTTCGGTTCCCTCTTAGGCATC 60
                ************************************************************
HWB1         61:TGCCTAATCTTGCAAATCCTAACAGGCCTGTTCTTAGCAATACATTACACATCAGACACA 120
HWB2         61:TGCCTAATCTTGCAAATCCTAACAGGCCTGTTCTTAGCAATACATTACACATCAGACACA 120
HCS          61:TGCCTAATCTTGCAAATCCTAACAGGCCTGTTCTTAGCAATACATTACACATCAGACACA 120
HC2          61:TGCCTAATCTTGCAAATCCTAACAGGCCTGTTCTTAGCAATACATTACACATCAGACACA 120
HC1          61:TGCCTAATCTTGCAAATCCTAACAGGCCTGTTCTTAGCAATACATTACACATCAGACACA 120
                ************************************************************
HWB1        121:ACAACAGCTTTCTCATCAGTTACACACATCTGTCGAGACGTAAATTACGGATGAGTTATT 180
HWB2        121:ACAACAGCTTTCTCATCAGTTACACACATCTGTCGAGACGTAAATTACGGATGAGTTATT 180
HCS         121:ACAACAGCTTTCTCATCAGTTACACACATCTGTCGAGACGTAAATTACGGATGAGTTATT 180
HC2         121:ACAACAGCTTTCTCATCAGTTACACACATCTGTCGAGACGTAAATTACGGATGAGTTATT 180
HC1         121:ACAACAGCTTTCTCATCAGTTACACACATCTGTCGAGACGTAAATTACGGATGAGTTATT 180
                ************************************************************
HWB1        181:CGCTACCTACATGCAAACGGAGCATCCATGTTCTTTATTTGCCTATTCATCCACGTAGGC 240
HWB2        181:CGCTACCTACATGCAAACGGAGCATCCATGTTCTTTATTTGCCTATTCATCCACGTAGGC 240
HCS         181:CGCTACCTACATGCAAACGGAGCATCCATGTTCTTTATTTGCCTATTCATCCACGTAGGC 240
HC2         181:CGCTACCTACATGCAAACGGAGCATCCATGTTCTTTATTTGCCTATTCATCCACGTAGGC 240
HC1         181:CGCTACCTACATGCAAACGGAGCATCCATGTTCTTTATTTGCCTATTCATCCACGTAGGC 240
                ************************************************************
HWB1        241:CGAGGCCTATACTACGGATCCTATATATTCCTAGAAACATGAAACATTGGAGTAGTCCTA 300
HWB2        241:CGAGGCCTATACTACGGATCCTATATATTCCTAGAAACATGAAACATTGGAGTAGTCCTA 300
HCS         241:CGAGGCCTATACTACGGATCCTATATATTCCTAGAAACATGAAACATTGGAGTAGTCCTA 300
HC2         241:CGAGGCCTATACTACGGATCCTATATATTCCTAGAAACATGAAACATTGGAGTAGTCCTA 300
HC1         241:CGAGGCCTATACTACGGATCCTATATATTCCTAGAAACATGAAACATTGGAGTAGTCCTA 300
                ************************************************************
HWB1        301:CTATTTACCGTTATAGCAACAGCCTTCATAGGCTACGTCCTGCCCTGAGGACAAATATCA 360
HWB2        301:CTATTTACCGTTATAGCAACAGCCTTCATAGGCTACGTCCTGCCCTGAGGACAAATATCA 360
HCS         301:CTATTTACCGTTATAGCAACAGCCTTCATAGGCTACGTCCTGCCCTGAGGACAAATATCA 360
HC2         301:CTATTTACCGTTATAGCAACAGCCTTCATAGGCTACGTCCTGCCCTGAGGACAAATATCA 360
HC1         301:CTATTTACCGTTATAGCAACAGCCTTCATAGGCTACGTCCTGCCCTGAGGACAAATATCA 360
                ************************************************************
```

Figure 4.4 1046bp of *Cyt* b gene sequences in 5 haplotypes in three pig populations

```
HWB1        361:TTCTGAGGAGCTACGGTCATCACAAATCTACTATCAGCTATCCCTTATATCGGAACAGAC 420
HWB2        361:TTCTGAGGAGCTACGGTCATCACAAATCTACTATCAGCTATCCCTTATATCGGAACAGAC 420
HCS         361:TTCTGAGGAGCTACGGTCATCACAAATCTACTATCAGCTATCCCTTATATCGGAACAGAC 420
HC2         361:TTCTGAGGAGCTACGGTCATCACAAATCTACTATCAGCTATCCCTTATATCGGAACAGAC 420
HC1         361:TTCTGAGGAGCTACGGTCATCACAAATCTACTATCAGCTATCCCTTATATCGGAACAGAC 420
                ************************************************************
HWB1        421:CTCGTAGAATGAATCTGAGGGGGCTTTTCCGTCGACAAAGCAACCCTCACACGATTCTTT 480
HWB2        421:CTCGTAGAATGAATCTGAGGGGGCTTTTCCGTCGACAAAGCAACCCTCACACGATTCTTC 480
HCS         421:CTCGTAGAATGAATCTGAGGGGGCTTTTCCGTCGACAAAGCAACCCTCACACGATTCTTC 480
HC2         421:CTCGTAGAATGAATCTGAGGGGGCTTTTCCGTCGACAAAGCAACCCTCACACGATTCTTC 480
HC1         421:CTCGTAGAATGAATCTGAGGGGGCTTTTCCGTCGACAAAGCAACCCTCACACGATTCTTC 480
                ************************************************************
HWB1        481:GCCTTTCACTTTATCCTGCCATTCATCATTACCGCCCTCGCAGCCGTACATCTCCTATTC 540
HWB2        481:GCCTTTCACTTTATCCTGCCATTCATCATTACCGCCCTCGCAGCCGTACATCTCCTATTC 540
HCS         481:GCCTTTCACTTTATCCTGCCATTCATCATTACCGCCCTCGCAGCCGTACATCTCCTATTC 540
HC2         481:GCCTTTCACTTTATCCTGCCATTCATCATTACCGCCCTCGCAGCCGTACATCTCCTATTC 540
HC1         481:GCCTTTCACTTTATCCTGCCATTCATCATTACCGCCCTCGCAGCCGTACATCTCCTATTC 540
                ************************************************************
HWB1        541:CTGCACGAAACCGGATCCAACAACCCTACCGGAATCTCATCAGACATAGACAAAATTCCA 600
HWB2        541:CTGCACGAAACCGGATCCAACAACCCTACCGGAATCTCATCAGACATAGACAAAATTCCA 600
HCS         541:CTGCACGAAACCGGATCCAACAACCCTACCGGAATCTCATCAGACATAGACAAAATTCCA 600
HC2         541:CTGCACGAAACCGGAACCAACAACCCTAACGGAATCTCTTCCGACCTAGACAAAATTCCA 600
HC1         541:CTGCACGAAACCGGATCCAACAACCCTACCGGAATCTCATCAGACATAGACAAAATTCCA 600
                *************** ************ ********* ** *** **************
HWB1        601:TTTCACCCATACTACACTATTAAAGACATTCTAGGGGCCTTATTTATAATACTAATCCTA 660
HWB2        601:TTTCACCCATACTACACTATTAAAGACATTCTAGGGGCCTTATTTATAATACTAATCCTA 660
HCS         601:TTTCACCCATACTACACTATTAAAGACATTCTAGGGGCCTTATTTATAATACTAATCCTA 660
HC2         601:TTTCACCCATACTACACTATTAAAGACATTCTAGGGGCCTTATTTATAATACTAATCCTA 660
HC1         601:TTTCACCCATACTACACTATTAAAGACATTCTAGGGGCCTTATTTATAATACTAATCCTA 660
                ************************************************************
HWB1        661:CTAATCCTTGTACTATTCTCACCAGACCTACTAGGAGACCCAGACAACTACACCCCAGCA 720
HWB2        661:CTAATCCTTGTACTATTCTCACCAGACCTACTAGGAGACCCAGACAACTACACCCCAGCA 720
HCS         661:CTAATCCTTGTACTATTCTCACCAGACCTACTAGGAGACCCAGACAACTACACCCCAGCA 720
HC2         661:CTAATCCTTGTACTATTCTCACCAGACCTACTAGGAGACCCAGACAACTACACCCCAGCA 720
HC1         661:CTAATCCTTGTACTATTCTCACCAGACCTACTAGGAGACCCAGACAACTACACCCCAGCA 720
                ************************************************************
HWB1        721:AACCCACTAAACACCCCACCCCATATTAAACCAGAATGATATTTCTTATTCGCCTACGCT 780
HWB2        721:AACCCACTAAACACCCCACCCCATATTAAACCAGAATGATATTTCTTATTCGCCTACGCT 780
HCS         721:AACCCACTAAACACCCCACCCCATATTAAACCAGAATGATATTTCTTATTCGCCTACGCT 780
HC2         721:AACCCACTAAACACCCCACCCCATATTAAACCAGAATGATATTTCTTATTCGCCTACGCT 780
HC1         721:AACCCACTAAACACCCCACCCCATATTAAACCAGAATGATATTTCTTATTCGCCTACGCT 780
                ************************************************************
```

Figure 4.4(Continued) 1046bp of *Cyt* b gene sequences in 5 haplotypes in three pig populations

```
HWB1        781:ATCCTACGTTCAATTCCTAATAAACTAGGTGGAGTGCTAGCTCTAGTAGCCTCCATCCTA 840
HWB2        781:ATCCTACGTTCAATTCCTAATAAACTAGGTGGAGTGCTAGCTCTAGTAGCCTCCATCCTA 840
HCS         781:ATCCTACGTTCAATTCCTAATAAACTAGGTGGAGTGCTAGCTCTAATAGCCTCCATCCTA 840
HC2         781:ATCCTACGTTCAATTCCTAATAAACTAGGTGGAGTGCTAGCTCTAATAGCCTCCATCCTA 840
HC1         781:ATCCTACGTTCAATTCCTAATAAACTAGGTGGAGTGCTAGCTCTAATAGCCTCCATCCTA 840
                ****************************************** **************
HWB1        841:ATCCTAATTTTAATGCCCATACTACACACATCCAAACAACGAAGCATAATATTTCGACCA 900
HWB2        841:ATCCTAATTTTAATGC:CATACTACACACATCCAAACAACGAAGCATAATATTTCGACCA 900
HCS         841:ATCCTAATTTTAATGCCCATACTACACACATCCAAACAACGAAGCATAATATTTCGACCA 900
HC2         841:ATCCTAATTTTAATGCCCATACTACACACATCCAAACAACGAAGCATAATATTTCGACCA 900
HC1         841:ATCCTAATTTTAATGCCCATACTACACACATCCAAACAACGAAGCATAATATTTCGACCA 960
                ************************************************************
HWB1        901:CTAAGTCAATGCCTATTCTGAATACTAGTAGCAGACCTCATTACACTAACATGAATTGGA 960
HWB2        901:CTAAGTCAATGCCTATTCTGAATACTAGTAGCAGACCTCATTACACTAACATGAATTGGA 960
HCS         901:CTAAGTCAATGCCTATTCTGAATACTAGTAGCAGACCTCATTACACTAACATGAATTGGA 960
HC2         901:CTAAGTCAATGCCTATTCTGAATACTAGTAGCAGACCTCATTACACTAACATGAATTGGA 960
HC1         901:CTAAGTCAATGCCTATTCTGAATACTAGTAGCAGACCTCATTACACTAACATGAATTGGA 960
                ************************************************************
HWB1        961:GGACAACCCGTAGAACACCCATTCATCATCATCGGCCAACTAGCCTCCATCTTATATTTC 1020
HWB2        961:GGACAACCCGTAGAACACCCATTCATCATCATCGGCCAACTAGCCTCCATCTTATATTTC 1020
HCS         961:GGACAACCCGTAGAACACCCATTCATCATCATCGGCCAACTAGCCTCCATCTTATATTTC 1020
HC2         961:GGACAACCCGTAGAACACCCATTCATCATCATCGGCCAACTAGCCTCCATCTTATATTTC 1020
HC1         961:GGACAACCCGTAGAACACCCATTCATCGTCATCGGCCAACTAGCCTCCATCTTATATTTC 1020
                *************************** ********************************
HWB1        1021:CTAATCATTCTAGTATTGATACCAAT 1046
HWB2        1021:CTAATCATTCTAGTATTGATACCAAT 1046
HCS         1021:CTAATCATTCTAGTATTGATACCAAT 1046
HC2         1021:CTAATCATTCTAGTATTGATACCAAT 1046
HC1         1021:CTAATCATTCTAGTATTGATACCAAT 1046
                 *************************
```

Figure 4.4 (Continued) 1046bp of *Cyt* b gene sequences in 5 haplotypes in three pig populations

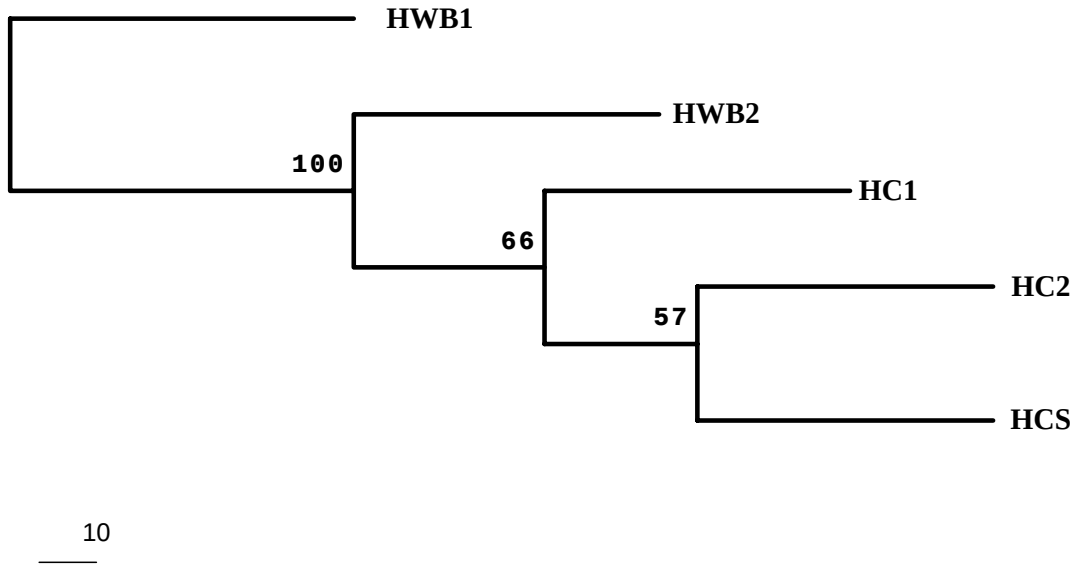

Figure 4.5 Phylogenetic tree constructed by Neighbor-Joining method based on five haplotypes in terms of 1046bp fragments of *Cyt* b gene of mtDNA. Bootstrap resampling was performed 1000 times.

HWB1: Haplotype 1 for Thai wild boars;

HWB2: Haplotype 2 for Thai wild boars;

HC1: Haplotype 1 for Chinese Qiabei Black pigs;

HC2: Haplotype 2 for Chinese Qianbei Black pigs;

HCS: Shared haplotype for Chinese Qianbei Black pigs and South Thai pigs

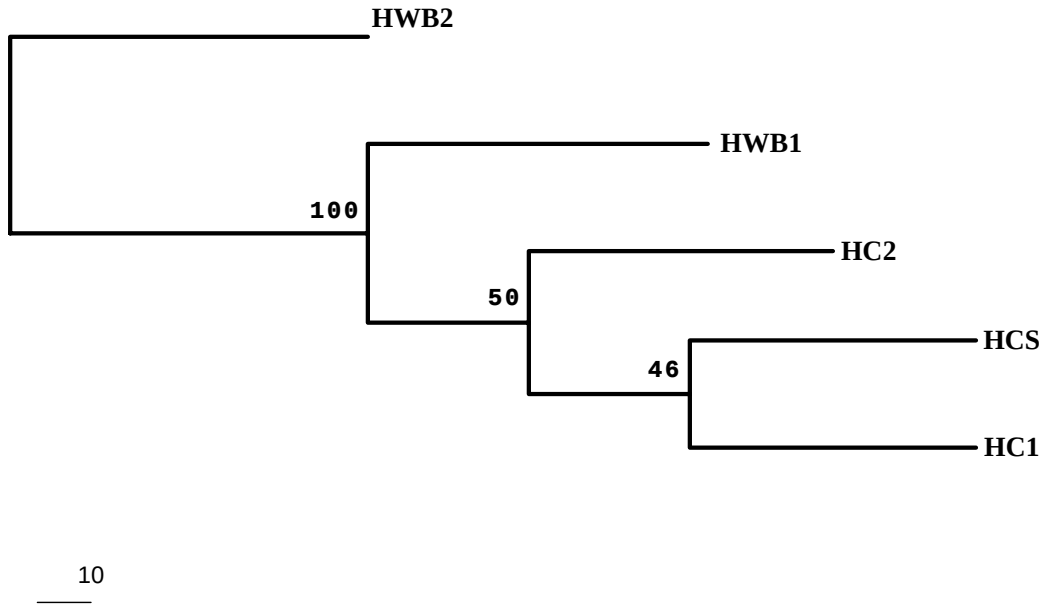

Figure 4.6 Phylogenetic tree constructed by UPGMA method based on five haplotypes in terms of 1046bp fragments of *Cyt* b gene of mtDNA. Bootstrap resampling was performed 1000 times.

HWB1: Haplotype 1 for Thai wild boars;

HWB2: Haplotype 2 for Thai wild boars;

HC1: Haplotype 1 for Chinese Qiabei Black pigs;

HC2: Haplotype 2 for Chinese Qianbei Black pigs;

HCS: Shared haplotype for Chinese Qianbei Black pigs and South Thai pigs

4.5 Conclusion

1046bp of *Cyt* b gene fragment (91.7% of whole *Cyt* b gene) from 17 samples based on three pig population were checked, a total of the five haplotypes and eight polymorphic nucleotide sites were detected. Only one haplotype (HCS) was found in south Thai pigs (ST population) from seven ST individuals, and shared the haplotype with the other CQB population, the haplotype frequency was relatively low. (A+T) content (57.2-57.3%) in all haplotypes were more than (G+C) content (42.7%-42.8%), and average contents for (A+T) and (G+C) were similar. Phylogenetic analysis was performed using Neighbor-Joining method and result indicated that CQB population had much closer genetic relationship with ST rather than WB population. This result is consistent with that study on phylogenetic relationship among same populations based on microsatellite markers stated in Chapter Ш. An conjecture could be made that Thai indigenous pigs were introduced from south or southwest of China.

4.6 References

Alex, C. *et al.* (2004). Estimating the frequency of Asian cytochrome B haplotypes in standard European and local Spanish pig breeds. **Genet. Sel. Evol.** 36: 97–104.

Alves, E., Ovilo, C., Rodrıguez, M.C., and Silio, L. (2003). Mitochondrial DNA sequence variation and phylogenetic relationships among Iberian pigs and other domestic and wild pig populations. **Anim. Genet.** 34: 319–24.

APHCA (Animal Production and Health Commission for Asia and the Pacific), FAO. (2002). The Livestock industries of Thailand. **RAP publication No.** 2002/23.

Bradley, D.G., MacHugh, D.E., Cunningham, P., and Loftus, R.T. (1996). Mitochondrial diversity and the origins of African and European cattle. **Proceedings of the National Academy of Sciences.** USA, 93: 5131–5.

Felsenstein J., (1993). PHYLIP(Phylogeny Inference Package) Version 3.67c, Department of Genetics, **University of Washington**, Seattle.

Excoffier, L. G. L., and Schneider, S. (2005). Arlequin ver. 3.0: An integrated software package for population genetics data analysis. **Evolutionary Bioinformatics Online**. 1: 47-50.

Giuffra, E., Kijas, J. M. H., Armager, V., Carlborg, O., Jeon, J.T., and Andersson, L. (2000). The origin of the domestic pig: independent domestication and subsequent introgression. **Genetics.** 154: 1785-1791.

Higgins, D. G., and Sharp, P. M. (1989). Fast and sensitive multiple sequence alignments on a microcomputer. **CABIOS.** 5: 151-153.

Higgins, D. G., and Sharp, P. M. (1988). CLUSTAL: a package for performing multiple sequence alignment on a microcomputer. **Gene**. 73: 237-244.

Hongo, H. *et al.* (2002). Variations in mitochondrial DNA of Vietnamese pigs: Relationships with Asian Domestic Pigs and Ruykuy Wild Boars. **Zoological Sci.** 19: 1329-1335.

Nei, M., and Li, W. (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. **Genetics.** 76: 5269-5273.

Nei, M. (1987). Molecular evolutionary genetics, **Columbia University Press.** New York. pp: 277.

Randi, E., V. L., and Diong, C.H. (1996). Evolutionary genetics of the suiformes as reconstructed using mtDNA sequencing. **Journal of Mammalian Evolution**. 3: 163-194.

Tanaka, Kazue. (1974). Morphological and serological studies on the native pigs in Thailand. **Report of the Society for Research on Native Livestock.** 6: 181-183.

Thompson, J. D., Gibson, T. J., Jeanmougin, F., and Higgins, D. G. (1997). The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. **Nucleic Acids Research**. 24: 4876-4882.

Tajima, F. (1989). Statistical methods to test for n nmucleotide mutation hypothesis by DNA polymorphism. **Genetics**. 123: 855-595.

Tamura, T., and Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control regeion of mitochondrial DNA. **Mol. Biol. Evol.** 10: 447-459.

Watanobe, T., Okumura, N., Ishiguro, N., Nakano, M., Matsui, A., Sahara, M., and Komatsu, M. (1999). Genetic relationship and distribution of the Japanese wild boar (*Sus scrofa leucomystax*) and Ryukyu wild boar (*Sus scrofa riukiuanus*) analyzed by mitochondrial DNA. **Mol Ecol**. 8: 1509–1512.

Yang, J. *et al.* (2003). Genetic Diversity Present within the Near-Complete mtDNA Genome of 17 Breeds of Indigenous Chinese Pigs. **Journal of Heredity.** 94(5): 381–385.

Zhang, Z. G. (1986). Pig Breeds in China. **Shanghai Scientific and Technical Publishers**, Shanghai.

CHAPTER V

ANALYSIS OF THE PHYLOGENETIC RELATIONSHIPS BETWEEN THAI PIGS AND EXOTIC PIG BREEDS BASED ON SEQUENCE POLYMORPHISM OF *Cyt b* GENE

5.1 Abstract

Four pig populations (NT, ST, CQB, WB) containing 36 pig samples were used to conduct phylogenetic analysis based on 1046bp of mtDNA *Cyt* b gene fragments. The results indicated that a total of 50 polymorphic sites are listed, 15 of 50 were transition substitutions, the other 34 were transversion substitutions, remaining one was transition/transversion occurrence simultaneously. 9 haplotypes (H1 to H9) were produced from 19 northeastern Thai pigs distributed in six provinces in Thailand. A multi-alignment analysis using sequences of 14 haplotypes indicated no repetitive sequences were detected, which means each haplotype was not identical to the other one. Average haplotype frequency was 38.9%. Phylogenetic trees based on Neighbor-Joining method and Maximum Parsimony method indicated accordant results, which are consistent with our inference that Thai native pig was probably originated from South or Southwest China. Phylogenetic trees was reconstructed using 14 haplotypes and 15 haplotypes representing exotic pig breeds from GenBank dada showed five Chinese domestic pig breeds including, Jinhua, Meishan, Xiang pig,

Qianbei black and South Thai pigs, together with one of north Thai lineages H1, were classified as a group, another group comprised of two European wild boar haplotype, Korean wild boar (HKR2), Japanese wild boar (HJP1), Yunnan wild boar and Duroc with higher bootstrap support values(from 57% to 100%). In subcluster A3, Chinese Wuzhishan and Large White were clustered into a branch with 55% bootstrap value. Two wild boars (HWB1and HWB2) in Thailand were not grouped as a clade with European wild boar, whereas were grouped into the same subcluster with a Japanese wild boar, and Vietnam wild boar, 5 Northeast Thai pigs (H3, H7, H5, H9, H8) were involved. Present results suggested that wild boars in Thailand had common ancestors with Southeast Asian wild boars. Further investigation is needed to confirm this point of view.

5. 2 Introduction

The origins and early exploration of Thai indigenous pigs remain unknown due to poor documentation or absence of records. In Chapter Ⅲ, we studied the phylogenetic relationships among several pig populations involving ST, WB, and CQB population. An inference was made that Thai indigenous pigs were probably originated from south or southwest China because they have identical haplotype sequences. This assumption was also made through our research on genetic diversity in terms of 12 microsatellite markers described in chapter Ⅲ.

In the light of the similarity in osteological characteristics, European and southeast Asian subspecies of the wild boar are thought to be the main ancestors of the domestic pig (Clutton-Brock, 1987). A significant differentiation between the European and Chinese domestic pigs has been revealed by mitochondrial DNA

analyses (Giuffra *et al*. 2000; Okumura *et al*. 2001; Watanobe *et al*. 2001; Kim *et al*. 2002). Tomowo *et al.* (2000) determined the mitochondrial *Cyt* b gene sequences (1140 bp) of four individuals of the wild boar and two individuals of the native domestic pig (*Sus scrofa*) in Laos and Vietnam. The *Cyt b* gene sequence of native domestic pigs in Laos and Vietnam was completely identical with that of Chinese Meishan pig, suggesting that both pigs had a late common ancestor.

However, little is known regarding study on phylogeny of Thai indigenous pig population. In chapter IV, a deduction was made that Thai indigenous pigs were probably introduced from south or southwest of China. But further comparative analysis related to sequences of haplotypes among Thai pigs and other Chinese domestic pigs are needed. Few reports on phylogenetic study of Thai indigenous pigs could be found. In particular, comparative phylogenetic study based on Thai pigs and some exotic pig breeds has not been reported.

In this chapter, comparative phylogenetic studies based on *Cyt* b gene fragments of mtDNA were conducted among two indigenous Thai pig populations including northeastern Thai pigs (NT) and southern Thai pigs (ST), a wild boar population(WB) in Thailand. Moreover, a Chinese Qianbei black pig breed and some exotic species were used to conduct the phylogenetic analyses.

5.3 Materials and Methods

5.3.1 Selection of samples

Table 5.1 Taxa used for molecular phylogenetic analysis from Genbank

Taxa (common name)	Name of halotype	Accession number	reference
Chinese Meishan	HMS	AB015082	Watanobe et al., 1999
Chinese Jinhua	HJH	AF486863	Yang et al., 2003
Chinese Rongchang	HRC	AF486860	Yang et al., 2003
Chinese Wuzhishan	HWZS	AF486867	Yang et al., 2003
Chinese Xiang	HX	AF486859	Yang et al., 2003
Large White	HLW	AB015079	Watanobe et al., 1999
Duroc	HDU	AB015080	Watanobe et al., 1999
European wild boar	HEW1	AB015083	Watanobe et al., 1999
European wild boar	HEW2	AB015082	Watanobe et al., 1999
Korean wild boar	HKR1	AY830171	Han et al., 2004
Korean wild boar	HKR2	AY692032	Han et al., 2004
Japanese wild boar	HJP1	AB015069	Watanobe et al., 1999
Japanese wild boar	HJP2	AB015065	Watanobe et al., 1999
Chinese Yunnan wild boar	HYN	DQ315599	Wu et al., 2006
Vietnam wild boar	HVN	DQ315603	Wu et al., 2006

Hair roots samples as described in Experiment IV were partly selected to conduct mitochondrial DNA analysis, but the sizes of samples were relatively smaller because of DNA quality and PCR effects. Finally, seven southern Thai pig samples (ST), eight Chinese Qianbei balck pig samples (CQB) and two wild boar samples (WB). In addition, nine haplotypes from nineteen fragment sequences of *Cyt* b gene of Northeastern Thai pigs were supplied by Miss Nitchanan Chukerd (2007). On the other hand, data from GenBank containing seven domestic pig breeds and eight wild boars were used for molecular phylogenetic analysis as well (Table. 5.1).

5.3.2 PCR for *Cyt* b gene and DNA purification

PCR method for ST, CQB and WB samples and PCR products purification has been described in chapter IV. Sequencing method for PCR products from NT samples are the same as that from ST and WB samples. Other data were taken from GenBank according to corresponding references.

5.3.3 Data analysis

GENETYX-WIN program version 3.1(Software Development Co. Ltd, Tokyo, Japan,) was applied to connect the forward DNA fragment and reverse DNA fragment, the final length was 1046bp, the majority of *Cyt* b gene sequences (91.7%) were aligned using GENETYX-WIN, of haplotypes were determined using CLUSTAL X program version 1.8 (Higgins et al., 1988). Levels of genetic variability were estimated as the number of polymorphic sites and haplotype diversity (*h*) (Nei, 1987) and nucleotide diversity (π) (Tajima, 1981) using MEGA 4.0 (Kumer *et al.*, 2004). After the sequences of all haplotypes were obtained, the restriction sites were

determined by using GENETYX-WIN program.

Pairwise genetic distances among mtDNA haplotypes were estimated across all populations using Kimura 2-Parameter (1993) model of evaluation using MEGA 4.0 (Kumer *et al.*, 2004). The computer package PHYLIP version 3.67 (Felsenstein, 1993) was employed to construct dendrogram. In order to compare the consistency of topology tree, both Neighbor-joining method and Maximum parsimony method were employed to construct dendrograms. The bootstrap method (Felsenstein, 1985) was applied to determine the confidence interval of each phylogeny from 1000 bootstrap repetitions. In present analysis, bootstrap value being lower than 50% did not show on tree branches.

5.4 Results and discussion

Part A: Analysis of phylogenetic relationships compared with NT pig population

5.4.1 Nucleotide variable site and sequence polymorphism

Table 5.3 indicates all the polymorphic sits in *Cyt* b gene of mtDNA based on 14 haplotypes representing four pig populations (ST, NT, CQB, and WB), sample size was 36. A total of 50 polymorphic sites are listed, 15 of them were transition substitutions, the other 34 were transversion substitutions, remaining one was transition/transversion occurrence simultaneously. Of all 14 haplotypes, a total of 9 haplotypes containing H1 to H9 were produced from 19 northeastern Thai pigs distributed in six provinces. A multi-alignment analysis was conducted using sequences of 14 haplotypes by means of program Clustal X (version 1.8), result indicted no repetitive sequences were detected (Figure 5.1), which means each

haplotype was not identical to the other one. In the other words, among all haplotypes based on four pig populations NT, ST, CQB, WB, only haplotype HCS was detected not only in CQB pigs but also in ST pigs, the remaining haplotypes such as HC1-HC2, H1-H9, HWB1-HWB2 occurred in single population. Average haplotype frequency was 38.9% (Table 5.2).

Table 5.2 Number and Distribution of haplotypes in 19 NT pig individuals

Haplotype	Number	Distribution(province)
H1	1	1Nahkon Phanom;
H2	7	1Loei, 3Sisaket, 3Mukdahan;
H3	5	1Sisaket, 2Surin, 2Lei;
H4	1	1Sisaket;
H5	1	1Surin;
H6	1	1Lei;
H7	1	1Lei;
H8	1	1Lei;
H9	1	1Nahkon Phanom

Source: Nitchanan

Table 5.3 Variable positions in *Cyt* b gene of mtDN

H	Variable position																								
	43	52	70	126	135	144	146	155	160	161	191	208	229	312	363	480	525	546	552	556	569	570	579	582	586
H5	G	T	T	C	A	A	A	G	G	T	A	A	A	T	C	C	C	C	C	T	C	C	A	A	A
H9	C	C	C	.	C	C	C	C	C	.	C	C	C	.	T	.	.	.	.	.	.	.	.	.	.
H3	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.
HWB1	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.
H4	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HC1	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HCS	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H1	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.
HC2	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	A	.	.	C	C
H2	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	T	.	.
HWB2	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H6	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H8	.	.	.	A	.	.	.	.	.	C	.	.	.	C	.	.	.	.	G	.	.	G	.	.	.
H7	.	.	.	A	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.

H = Haplotype

Table 5.3 (Continued) Variable positions in *Cyt* b gene of mtDNA from four pig populations

H	Variable position																								
	631	691	738	757	765	774	784	786	797	807	810	826	838	862	880	911	913	918	925	943	957	988	1000	1013	1015
H5	C	C	A	T	C	C	C	A	C	A	T	G	C	C	C	G	C	C	C	A	T	A	C	T	T
H9	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H3	.	.	..	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HWB 1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.
HC1	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.
HCS	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.
H1	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.
HC2	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.
H2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A
HWB 2	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	A	.	T	.	.	.	.	.	.	A
H6	T	T	.	G	T	T	T	.	T	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H8	.	.	C	.	.	.	G	G	A	G	G	.	T	A	G	.	T	.	G	C	G	.	G	A	.
H7																									

H = Haplotype

Table 5.4 Pairwise genetic distance based on fourteen haplotypes using Timura 2-parameter method.

	H5	**H9**	**H3**	**HWB1**	**H4**	**HC1**	**HCS**	**H1**	**HC2**	**H2**	**HWB2**	**H6**	**H8**	**H7**
H5														
H9	0.012													
H3	0.001	0.013												
HWB1	0.002	0.014	0.001											
H4	0.002	0.014	0.001	0.002										
HC1	0.003	0.014	0.002	0.003	0.003									
HCS	0.002	0.014	0.001	0.002	0.002	0.001								
H1	0.003	0.014	0.002	0.003	0.003	0.002	0.001							
HC2	0.007	0.018	0.006	0.007	0.007	0.006	0.005	0.006						
H2	0.003	0.014	0.002	0.003	0.003	0.002	0.001	0.002	0.006					
HWB2	0.002	0.014	0.001	0.002	0.002	0.003	0.002	0.003	0.007	0.003				
H6	0.005	0.016	0.014	0.005	0.004	0.004	0.003	0.004	0.008	0.004	0.003			
H8	0.014	0.025	0.013	0.014	0.014	0.014	0.014	0.014	0.017	0.013	0.014	0.016		
H7	0.016	0.028	0.015	0.016	0.016	0.017	0.016	0.017	0.021	0.017	0.016	0.019	0.022	

5.4.2 Phylogenetic tree based on the difference of haplotypes

Both Neighbor-Joining (NJ) method and maximum parsimony method were employed to construct the phylogenetic tree based on 14 haplotypes from using 36 individuals representing four pig populations (NT, ST, CQB, and WB). Similar results were detected from two kinds of tree constructing methods (Figure 5.2).

In Figure 5.1, 1046bp sequence fragments of mtDNA from four pig populations were classified into two major clusters, H7 and H8 were clustered into a lineage, the other four pig populations were clustered into a lineage consisting of 34 sequences, including those from 7 ST pigs, 8 CQB pigs, 2 WB pigs, and 17 from NT pigs. Haplotype HCS only representing ST pigs was classified as a subcluster with HC2 with 33% bootstrap values. This lineage also included H1, HC1, H2, HWB2 and H6. H4, HWB1, H3, H5, and H9 were classified as a subcluster. This means that ST pig population and Chinese pig CQB had close genetic correlation. The wild boar haplotype HWB1 and HWB2 were not classified into one clade. We noticed that H5 and H9 were clustered into the same group with 73% bootstrap value, though they are from different two provinces Surin and Nakon Phanom in Thailand (Table 5.2), there was closer genetic relationship between these two haplotypes. Another two haplotypes H7 and H8 were clustered into the same branch with a high bootstrap support value (86%). From geographic position, both H7 and H8 located on Lei province, maybe they were introduced from Laos. It can show there was closer genetic correlation between these two haplotypes.

Phylogenetic tree was also constructed using maximum parsimony method based on 1046bp of mtDNA *Cyt* b gene sequences of 14 haplotypes from four pig populations**.** The result showed a similar topologic structure compared to Figure 5.1.

Figure 5.2 also contains two clusters; H7 and H8 were classified as a cluster with a 80 % bootstrap value, other cluster comprised of 34 sequences, including those from 7 ST pigs, 8 CQB pigs, 2 WB pigs, and 17 native pigs from NT pigs. There was a slightly difference that haplotype HC2 did not classified as same branch with HCS while clustered with haplotype H1, and then HC1 and HCS, together with the other haplotypes including H2, H6, H3, H4 and two wild boars. The similar result to Figure 5.1, H5 and H9 were clustered into the same lineage with 80% bootstrap value.

Phylogenetic trees based on Neighbor-Joining method and Maximum Parsimony method indicated the accordant results. First, South Thai pigs and Chinese Qianbei black pigs were clustered the same branch; it suggests that there was close genetic relationship between these two pig populations. This result supports the conjectures given in chapter Π and chapter ΙΙΙ. Second, it may given a conclusion that haplotype H5 and H9, H7 and H8 may be the same lineages because they were classified the same group with a higher bootstrap values thought the number of haplotypes maybe not enough. In fact, it is not well documented that how many native pig breeds in Thailand, but molecular data presented here may indicate at least some lineages could be classified within Thai indigenous pigs. Further studies regarding molecular phylogeny in terms of Thai pig breeds are needed.

Previous studies provided comprehensive molecular analyses on genetic relationship between domestic pigs and wild boars (Giuffra *et* al. 2000; Okumura *et al*. 2001; Watanobe *et al.* 2001; Kim *et al.* 2002., Tomowo *et al*., 2000), but documents are extremely limited regarding mtDNA sequence analysis in Thai pigs, CQB pigs, and WB pigs, so it could not be described that whether Thai indigenous pig

population belongs to Asian haplotypes or European haplotypes. This work is quite necessary; it will be discussed in part B.

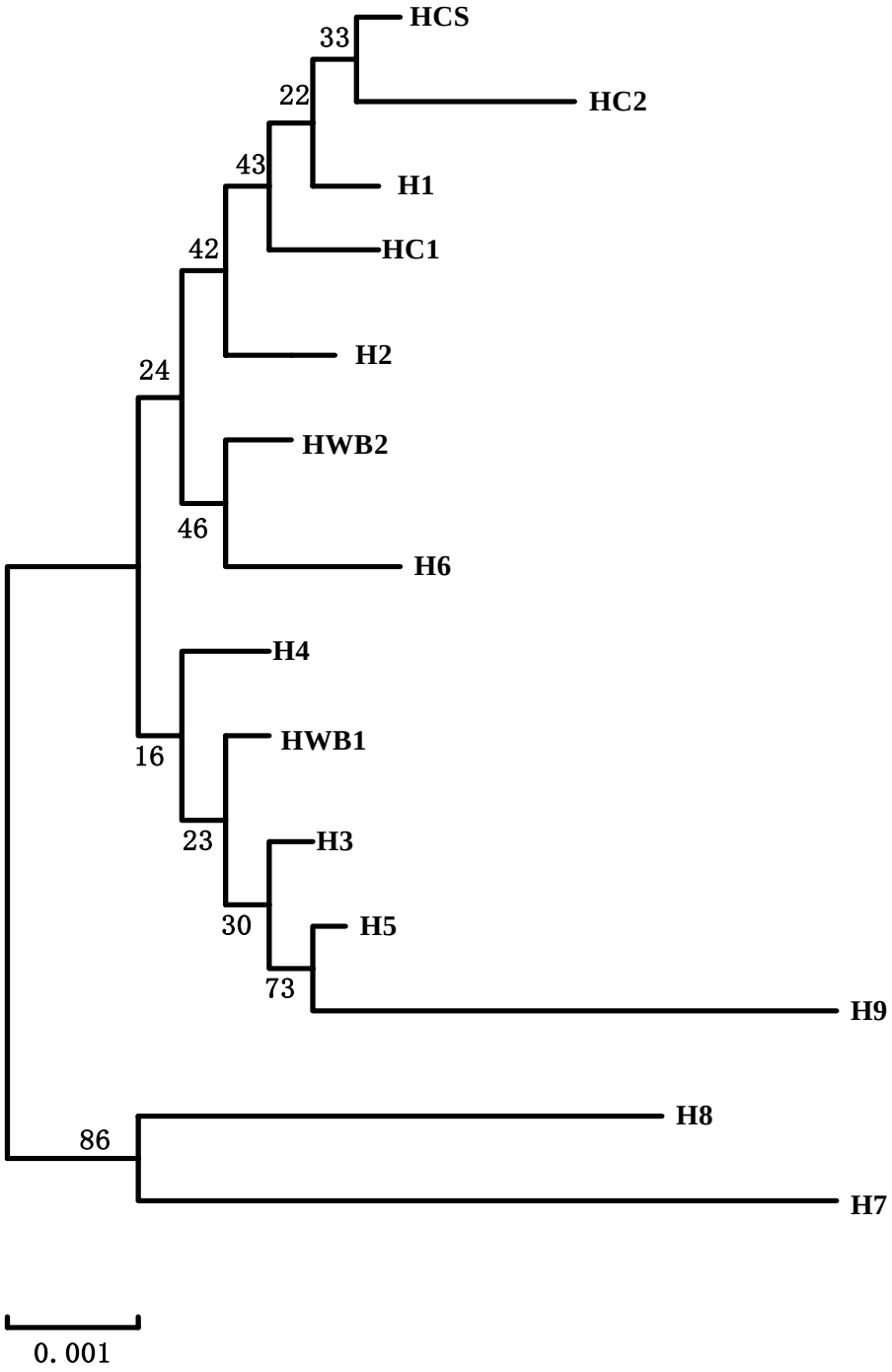

Figure 5.1 Neighbor-Joining (NJ) tree was constructed based on 14 haplotypes using 1046bp of mtDNA *Cyt* b gene sequences from four pig populations (NT, ST, CQB, WB). The numbers at the nodes are the bootstrap support based on 1000 replicates.

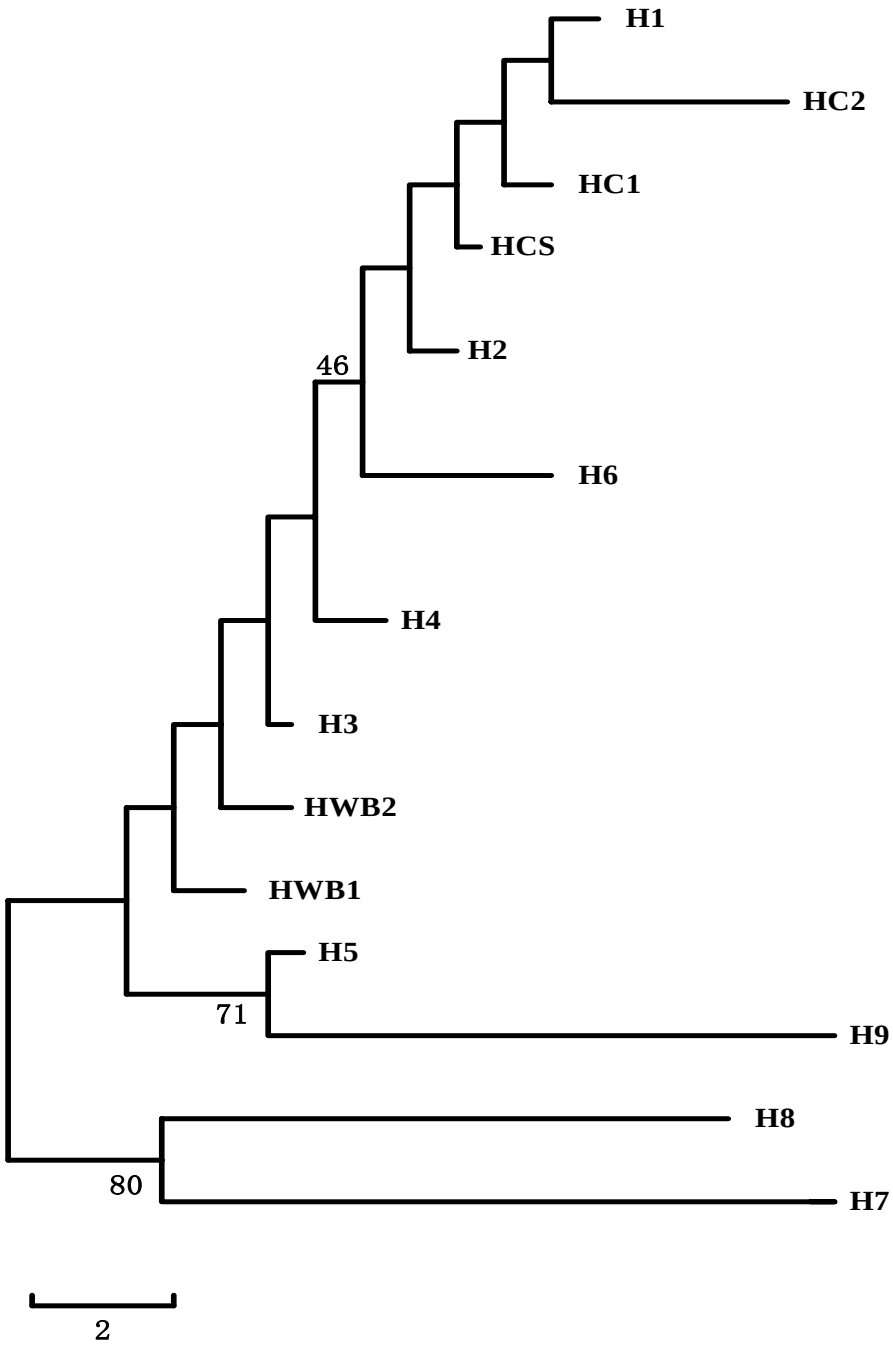

Figure 5.2 Maximum parsimony (MP) tree was constructed based on 14 haplotypes using 1046bp of mtDNA *Cyt* b gene sequences from four pig populations (NT, ST, CQB, WB). The numbers at the nodes are the bootstrap support based on 1000 replicates.

Part B: Analysis of phylogenetic relationships compared with 15 exotic pig populations

Alignment was performed using 14 haplotypes of *Cyt* b gene fragments representing Thai pig population and Chinese pig populations described in Part A and 15 haplotypes representing south and southwest Chinese domestic pigs, some Asian wild boars, and European wild boars (data from GenBank, Table 5.1). A total of 86 Variable sites are listed (Table 5.5). Comparing their haplotype sequences with our data, no any identical sequence could be found.

Data taking from GenBank contained south Chinese pig breeds, southwest Chinese pig breeds, central Chinese pig breeds, Asian wild boars, and European wild boars. Among these breeds, Rongchang pigs originated from Sichuan province of China (haplotype HRC, AF486860, and was divided into the same type Ⅴ with Qianbei balck pigs according to Zhang (1986), Xiang pig is from Guizhou province. In terms of geographic position, these provinces including Yunnan and Vietnam are close to the others. Data presented here did not show a high correlation between the genetic classification and geographic distribution of Thai pigs and Asian pig breeds.

Phylogenetic tree was reconstructed based on Neighbor-Joining method among 29 (including out-group haplotype HKR1) different haplotypes. All sequences of haplotypes were classified into two major groups (group A1, A2, A3, and group B; Figure 5.4). Group A composed of subcluster A1, A2, and A3, group B only consisted of a Japanese wild boar haplotype 1. Group A1 was composed of six *Cyt* b sequences of Chinese domestic pigs including HX, HC1, HC2, HRC, HMS, and HJH, a Northeast Thai pig and a shared haplotype HCS, result suggest Thai indigenous pig has closer genetic relationship. Subcluster A2 consists of sequenes of three Asian wild

boar haplotypes (HKR2, HJP1, HYN) and two European wild boar haplotype (HEW1, and HEW2) with higher bootstrap values, a European domestic pig was also included. Subcluster A3 consists of most of NT haplotypes, two Thai wild boar haplotype (HWB1 and HWB2) a Vietnam wild boar HVN, a European domestic pig Large white HLW.

Phylogenetic analysis presented here, clearly indicating close genetic correlation of Thai indigeous pigs with Chinese pigs, are consistent with our inference that Thai pigs is probably originated from south or southwest China described in Chapter III and chapter IV. But in this topological structure shown in Figure 5.4 South Thai pigs had closer genetic relationship with Chinese Xiang pigs and CQB pigs. The Asian haplotypes found in European pigs has been revealed (Giuffra *et al*., 2000; Kim *et al*., 2002; Fang and Andersson, 2006). In general, there was an agreement that some breeds with a well-documented were affected by Asian pigs. For instance, Berkshare and Large White exhibited a high frequency of Asian mtDNA haplotypes. Accordingly, the presence of Asian haplotypes in two Spanish pig breeds, Manchado de Jabugo and Negro Canario, was consistent with the known introgression of Tamworth and Black pigs, carrying Asian haplotypes, from United Kingdom to Spain in 1980). (http://www.tihohannover.de/einricht/zucht/eaap/index.htm). In our study, haplotype HCS representing South Thai pigs was not clustered as one clade with European wild boars. In contrast, HCS was grouped into a clade with main south Chinese pig or southwest Chinese pig (HX, HRC), identical mtDNA sequence with Chinese Qianbei Black pigs also indicated it should be classified as Asian haplotype. Certainly, small sampling size of ST population may not confirm ST only has one haplotype, further studies when increasing sampling size are needed.

A poor documentation is related to origin of wild boars living in Thailand, but there is an agreement that European and Southeast Asian subspecies of the wild boar are thought to be the main contributors to the genetic makeup of the domestic pig (Clutton-Brock 1987). It has been proposed that Chinese pigs were domesticated from local wild boar populations in several different regions, and the south China wild boar (S. scrofa chirodontus) and the north China wild boar (S. scrofa moupiensis) are considered the two main ancestors (Zhang, 1986). No mtDNA haplotypes of European wild boars were detected in Asian pigs. However, the contradiction result could be found that a European wild pig haplotype (EWB1) was the member of Asian clade. Several factors can lead to this result. In present study, two wild boars (HWB1and HWB2) in Thailand were not grouped into wild boar haplotypes A2. Conversely, they seemed to close to NT pig population. There was not enough sample size. Therefore, further studies are necessary to infer where they came from.

Table 5.5 Comparison of variable position using 1046bp of mtDNA *Cyt* b gene fragment with exotic 15 pig breeds

H	Variable positions																						
	25	**43**	**52**	**70**	**90**	**126**	**135**	**144**	**146**	**150**	**155**	**159**	**160**	**161**	**165**	**186**	**191**	**208**	**210**	**229**	**246**	**274**
HRC	T	G	T	T	G	A	A	A	A	C	G	C	G	T	T	C	A	A	G	A	C	G
HX	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HJH	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HMS	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HCS	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HC2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HC1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HEW1	C	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	T	.
HEW2	C	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	T	.
HDU	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	T	.	.	A	.	T	.
HYN		.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	A	.	T	A
HJP1		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.
HKR2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.
HWZS	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HLW	.			.	.	.				.	.	T	.		.	.		.	.	.	.	.
HWB2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HWB1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HKR1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HJP2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HVN	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H5	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.
H9	.	.	.	C	.	C	.	.	C	.	C	.	.	.	.	.	.	C	.	C	.	.
H4	.	C	C	.	.	.	C	C	.	.	.	.	.	.	.	.	C	.	.	.	.	.
H7	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H8	.	.	.	.	.	.	.	.	.	.	.	.	C	.			.	.	.	.	.	.
	.	.	.				.	.						C			.					

H= Haplotype

Table 5.5 (Continued) Comparison of variable position using 1046bp of mtDNA *Cyt* b gene fragment with exotic 15 pig breeds

H	Variable positions																					
	312	**316**	**337**	**363**	**375**	**387**	**411**	**414**	**480**	**484**	**486**	**525**	**546**	**548**	**552**	**556**	**561**	**569**	**570**	**579**	**581**	**582**
HRC	T	G	G	C	G	T	C	A	C	T	T	C	C	A	C	T	C	A	C	A	C	A
HX	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HJH	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HMS	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HCS	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HC2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	..	.	T	.	C
H1	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.
HC1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.
HEW1	.	A	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.
HEW2	.	A	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.
HDU	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HYN	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.
HJP1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HKR2	.	.	.	.	.	.	.	G	.	C	.	.	.	G	.	.	.	.	.	.	G	.
HWZS	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HLW	.	.	.	.	A	.	T	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.
HWB2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HWB1	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.
HKR1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HJP2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HVN	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H9	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H7		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H8	C C	.	.	T	.	.	.	.	.	.	.	G	.	.	G	.	.		.	.	.	.

H= Haplotype

Table 5.5 (Continued) Comparison of variable position using 1046bp of mtDNA *Cyt* b gene fragment with exotic 15 pig breeds.

H	Variable positions																						
	586	**592**	**631**	**636**	**637**	**640**	**691**	**711**	**738**	**752**	**757**	**765**	**774**	**783**	**784**	**786**	**789**	**797**	**807**	**810**	**817**	**819**
HRC	A	A	C	G	G	T	C	C	A	C	T	C	C	C	C	A	T	C	A	T	C	A
HX	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HJH	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HMS	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HCS	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HC2		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H1	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HC1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HEW1	.	.	.	A	A	.	.	T	.	.	.	.	.	T	.	.	.	.	.	.	T	G
HEW2	.	.	.	A	A	.	.	T	.	.	.	.	.	T	.	.	.	.	.	.	T	G
HDU	.	.	.	A	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	T	G
HYN	.	.	.	A	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	T	.
HJP1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.
HKR2	.	G	.	.	.	G	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.
HWZS	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HLW	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HWB2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HWB1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HKR1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	..	.	.	.	.	.
H3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HJP2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HVN	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H9	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H7	.	.	.	.	.	.	.	.	C	.	.	.	.	.	G	G	.	A	G	G	.	.
H8	.	.	T	.	.	.	T	.	.	.	G	T	T	.	T	.	C	T	.	G	.	.
	.																					

H= Haplotype

Table 5.5 (Continued) Comparison of variable position using 1046bp of mtDNA *Cyt* b gene fragment with exotic 15 pig breeds

H	Variable positions																					
	822	**826**	**838**	**862**	**867**	**880**	**883**	**894**	**899**	**911**	**913**	**918**	**925**	**943**	**957**	**981**	**988**	**993**	**1000**	**1013**	**1015**	**1017**
HRC	T	A	C	C	C	C	A	T	C	G	C	C	C	A	T	A	A	C	C	T	T	T
HX	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HJH	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HMS	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HCS	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HC2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HC1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.
H2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HEW1	.	G	.	.	.	.	.	C	.	.	.	.	.	.	.	G	.	.	.	.	.	C
HEW2	.	G	.	.	.	.	.	C	.	.	.	.	.	.	.	G	.	.	.	.	.	C
HDU	C	G	.	.	.	.	G	.	.	.	.	.	.	.	.	G	.	.	.	.	.	C
HYN	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HJP1	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HKR2	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.
HWZS	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HLW	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.
HWB2	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.
H6	.	.	.	.	.	.	.	.	.	A	.	T	.	.	.	.	.	.	.	.	A	.
HWB1	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HKR1	.	G	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H3	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HJP2	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HVN	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H5	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H9	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H4	.	G	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.
H7	.	G	T	A	.	G	.	.	.	.	T	.	G	C	G	.	.	.	G	A	.	.
H8	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

H = Haplotype

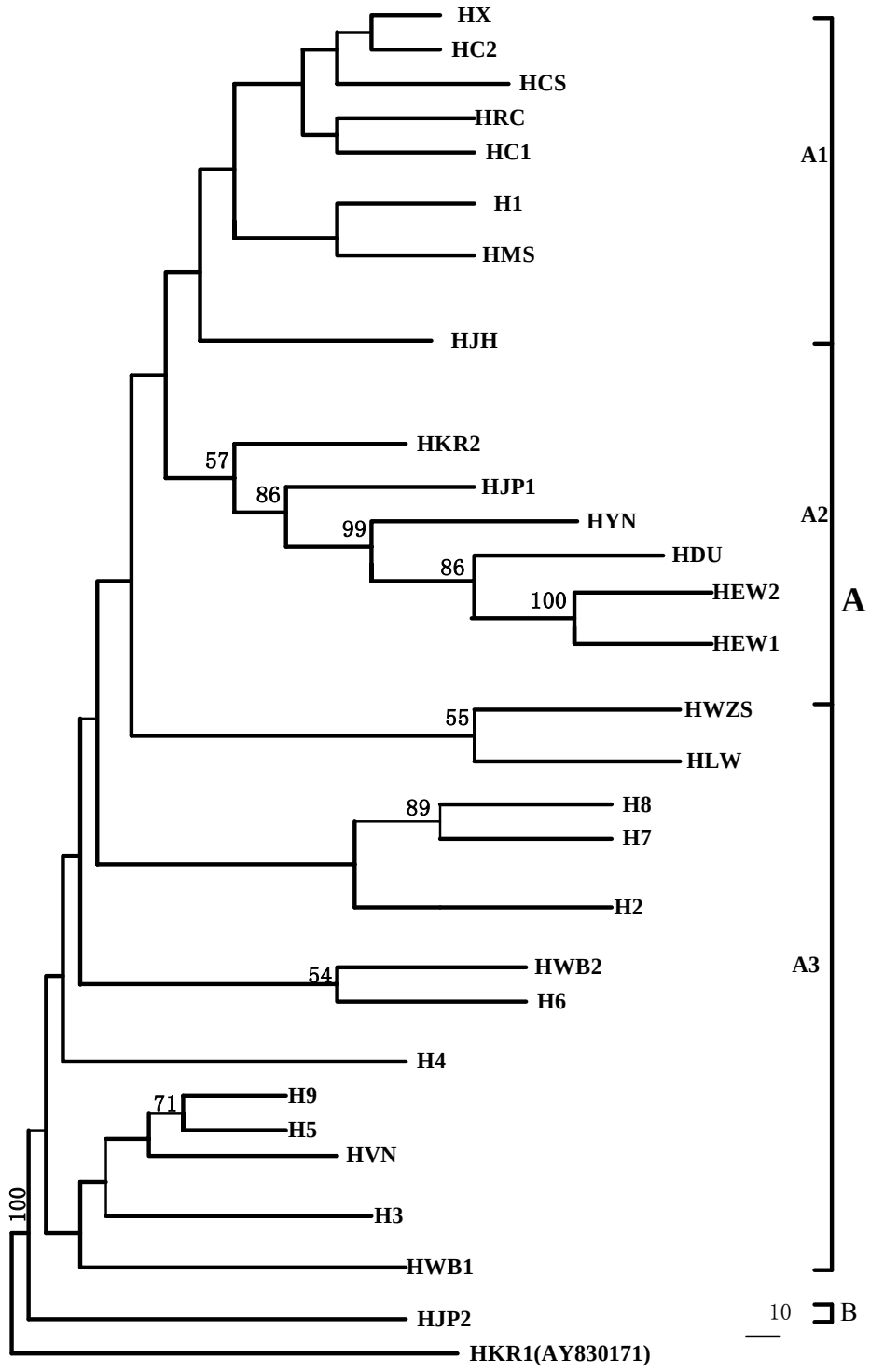

Figure 5.4 Neighbor-joining tree (NJ) was constructed among 29 haplotypes including four pig populations (NT, ST, CQB, WB) and exotic pig populations. HKR1 sequence was used as an out-group. Unlabelled nodes received less than 50% bootstrap support.

5.5 Conclusion

Phylogenetic trees based on Neighbor-Joining method and Maximum Parsimony method indicated accordant results, are consistent with the inference in Chapter IV that South Thai pig was probably originated from South or Southwest China. South Thai pigs and five Chinese domestic pig breeds including, Jinhua, Meishan, Xiang pig, Qianbei black and three North Thai pigs had closer genetic relationships. Two wild boars (HWB1and HWB2) living in Thailand were not grouped as a clade with European wild boar, whereas were grouped into the same subcluster with a Japanese wild boar, a Korean wild boar, and Vietnam wild boar, some Northeast Thai pigs were involved. Our results suggested that wild boars living in Thailand had common ancestors with Southeast Asian wild boars.

5.6 References

Clutton-Brock, J. (1987). Man-made animals: pigs. In a natural history of domesticated mammals, Cambridge, UK: **Cambridge University Press.** pp: 71–77.

Fang, M., and Andersson, L. (2006). Mitochondrial diversity in European and Chinese pigs is consistent with population expansions that occurred prior to domestication. **Proc. R. Soc. B.** 273: 1803–1810.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. **Evolution.** 39: 783-791.

Giuffra, E., H. Kijas, J. M. Arma, V., Carlborg, O. Jeon, J.T. and Andersson, L. (2000). The origin of the domestic pig: independent domestication and subsequent introgression. **Genetics.** 154: 1785-91.

Han, S. H., Cho, I. C., Song, J. H., Oh, J., M. C., and Oh, M.Y. (2004). Phylogenetic relationship of Korean wild boars based on mitochondrial DNA Cyt b gene. Submitted to NCBI (21-JUL-2004).

Kim, K. I., Lee, J. H., Li, K., Zhang, Y. P., Lee, S. S., Gongora, J. and Moran, C. (2002). Phylogenetic relationships of Asian and European pig breeds determined by mitochondrial DNA D-loop sequence polymorphism. **Anim. Genet.** 33: 19–25.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequence. **Journal of Molecular Evolution.** 16: 111-120.

Okumura, N. *et al.* (2001). Genetic relationship amongst the major non-coding regions of mitochondrial DNAs in wild boars and several breeds of domesticated pigs. **Anim. Genet.** 32: 139–147.

Tomowo, O. *et al.* (2000). Molecular Phylogenetic Analysis of the Wild Boars and Native Domestic Pigs in Lao. **Rep. Soc. Res.** Native Livestock. 18: 149-158.

Watanobe, T. *et al.* (1999). Genetic relationship and distribution of the Japanese wild boar (*Sus scrofa leucomystax*) and Ryukyu wild boar (*Susscrofa riukiuanus*) analysed by mitochondrial DNA. **Molecular Ecology.** 8: 1509–1512.

Wu, G. S., Pang, J. F., and Zhang, Y. P. (2006). The molecular phylogeny and phylogeography of Suidae. **Zoological Research**. 27(2): 197-201.

Yang, J. *et al.* (2003). Genetic Diversity Present within the Near-Complete mtDNA Genome of 17 Breeds of Indigenous Chinese Pigs. **Journal of Heredity.** 94 (5):381–385.

CHAPTER VI

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

This dissertation mainly focuses on study of genetic diversity among several indigenous Thai pig populations and a Chinese pig population based on microsatellite markers and polymorphism of mtDNA *Cyt* b gene. The conclusion can be stated as follows,

1. DNA quality and concentrations from blood and hair roots were compared. Results suggested that DNA taken from 100 or 200 pig hair roots could be used for PCR reaction based on microsatelite loci, 2.5 ng/μL and 5 ng/μL of DNA template concentration could obtain PCR products.

2. Thai indigenous pig population had high heterozygosity and exhibited a high genetic diversity compared with some Chinese native pig breeds, European pig breeds and some Asian pigs such as indigenous pigs from Indian and Korean native pigs. A UPGMA tree based on Nei's D_A standard genetic distances indicated that Chinese Qianbei Black pigs (CQB) and two Thai indigenous pig populations (NT, ST) were clustered into the same branches with a 100% of bootstrap support value. From current results, Thai native pigs population maybe originate from southwest or south

of China. These findings could be used as genetic information and further genetic improvement of Thai indigenous pigs.

3. Five haplotpyes and eight polymorphic nucleotide sites were detected from 1046bp of *Cyt* b gene fragment representing 17 samples of three pig populations. Only one haplotype (HCS) was found in South Thai pigs, and shared the haplotype with the five Chinese Qianbei black individuals. A inference could be made that ST pigs and CQB pigs have common ancestor.

4. Phylogenetic analysis on the base of *Cyt* b gene fragments indicated that south Thai pigs had much closer genetic relationship with Chinese Qianbei black pigs, which was consistent with that study on phylogenetic relationship among same populations based on microsatellite markers. This result supported the inference that Thai pigs might have the same origin as pigs of south or southeast China.

5. Phylogenetic analysis on base of *Cyt* b gene fragments using exotic pig breeds indicated South Thai pigs and five Chinese domestic pig breeds including, Jinhua, Rongchang, Meishan, Xiang pig, Qianbei black and one Northeast Thai pigs had closer genetic relationships. Two wild boars in Thailand were grouped into the same subcluster with a Japanese wild boar, and Vietnam wild boar, most of Northeast Thai pigs were involved. Data suggested that wild boars in Thailand probably had common ancestors with Southeast Asian wild boars.

6. It will be reliable if we can add to select north or central areas in Thailand as sample sites. Only one province in south of Thailand was selected as sampling site seems to be lack of representative. Sample size was relatively small, particularly in mtDNA research. Further studied are necessary to confirm our inference.

6.2 Recommendation

It is clear from result that Thai indigenous pig population had high heterozygosity and exhibited a high genetic diversity compared with some Chinese native pig breeds and other European species. There still exists a relatively large indigenous pig population. But reduction of Thai pigs in number has been increasing the possibility to disappear. In addition, low haplotype frequency also gives us an important implication that genetic diversity of Thai pigs has been decreasing. Thus, relative conservation strategy should be made to protect its genetic diversity.

Although there was not systematic classification regarding Thai indigenous pigs, microsatellite variations and phylogenetic analysis indicated the differences exist among Thai indigenous pigs from different areas. It is recommended that type or lineage classification is essential in order to identify their morphological or genetic variations.

In addition, the further studies with respect to mtDNA sequence need to be conducted to confirm origin of Thai indigenous pigs including wild boars by comparing pig populations from other regions of Thailand, some other Chinese pig breeds and introduced pig populations.

APPENDIX A

INFORMATION OF 27 PAIRS OF MICROSATELLITE MARKERS RECCOMMENDED BY ISAG/FAO IN 2004

Table A 1 Information of 27 pairs of microsatellite markers recommended by ISAG/FAO in 2004 (swine)

Mks	**Sequence of primers (5'-3')**	**Chrs**	**Ann. Temp./ $Mgcl_2$ (mM)**	**Size allele(bp)**
CGA	ATAGACATTATGTCCGTTGCTGAT GAACTTTCACATCCCTAAGGTCGT	1p	62/1.5	250-320
S0101	GAATGCAAAGAGTTCAGTGTAGG GTCTCCCTCACACTTACCGCAG	7	60 / 1.5	197-216
S0215	TAGGCTCAGACCCTGCTGCAT TGGGAGGCTGAAGGATTGGGT	13	55 / 4.0	135-169
S0355	TCTGGCTCCTACACTCCTTCTTGATG TTGGGTGGGTGCTGAAAAATAGGA	15	55/4.0	243-277
SW911	CTCAGTTCTTTGGGACTGAACC CATCTGTGGAAAAAAAAAGCC	9	60 / 1.5	153-177
SW936	TCTGGAGCTAGCATAAGTGCC GTGCAAGTACACATGCAGGG	15	58 / 1.5	80-117
S0068	AGTGGTCTCTCTCCCTCTTGCT CCTTCAACCTTTGAGCAAGAAC	13	62 / 1.5	211-260
SW632	ATCAGAACAGTGCGCCGT TTTGAAAATGGGGTGTTTCC	7	58 / 1.5	159-180
SW24	CTTTGGGTGGAGTGTGTGC ATCCAAATGCTGCAAGCG	17	58 / 1.5	96-211
S0227	GATCCATTTATAATTTTAGCACAAAGT GCATGGTGTGATGCTATGTCAAGC	4	55 / 4.0	231-256
S0225	GCTAATGCCAGAGAAATGCAGA CAGGTGGAAAGAATGGAATGAA	8	55 / 4.0	170-196
SW122	TTGTCTTTTTATTTTGCTTTTGG CAAAAAAGGCAAAAGATTGACA	6	58 / 1.5	110-122
S0090	CCAAGACTGCCTTGTAGGTGAATA GCTATCAAGTATTGTACCATTAGG	12	58 / 1.5	244-251
S0226	GCACTTTTAACTTTCATGATACTCC GGTTAAACTTTTNCCCCAATAC	2q	55 / 4.0	181-105

Mks = Markers; Chrs = chromosomes; Ann Temp.= Annealing Temperature.

Table A1 (Continued) Information of 27 pairs of microsatellite markers recommended by ISAG/FAO in 2004 (swine)

Mks	**Sequence of primers (5'-3')**	**Chrs**	**Ann. Temp. /Mgcl$_2$ (mM)**	**Size allele(bp)**
S0178	TAGCCTGGGAACCTCCACACGCTG GGCACCAGGAATCTGCAATCCAGT	8	58 / 1.5	110-124
S0005	TCCTTCCCTCCTGGTAACTA GCACTTCCTGATTCTGGGTA	5	58 / 1.5	205-248
S0386	TCCTGGGTCTTATTTTCTA TTTTTATCTCCAACAGTAT	11	48 / 3.0	15-174
SW72	TGAGAGGTCAGTTACAGAAGACC GATCCTCCTCCAAATCCCAT	3p	58 / 1.5	100-16
S0002	GAAGCCCAAAGAGACAACTGC GTTCTTTACCCACTGAGCCA	3q	62 / 1.5	190-216
SW857	AGAAATTAGTGCCTCAAATTGG AAACCATTAAGTCCCTAGCAAA	14	58 / 1.5	144-160
S0026	GCACTTTTAACTTTCATGATACTCC GGTTAAACTTTTNCCCCAATACA	16	55 / 4.0	92-106
IGF1	GCTTGGATGGACCATGTTG CATATTTTTCTGCATAACTTGAACCT	5	58 / 1.5	197-209
S0155	TGTTCTCTGTTTCTCCTCTGTTTG AAAGTGGAAAGAGTCAATGGCTAT	1q	55 / 1.5	150-166
SW240	TGGGTTGAAAGATTTCCCAA GGAGTCAGTACTTTGGCTTGA	2p	58 / 1.5	96-115
SW951	TTTCACAACTCTGGCACCAG GATCGTGCCCAAATGGAC	10	58 / 1.5	125-133
S0228	GGCATAGGCTGGCAGCAACA AGCCCACCTCATCTTATCTACACT	6	55 / 4.0	222-249
S0218	GTGTAGGCTGGCGGTTGT CCCTGAAACCTAAAGCAAAG	X	55 / 2.0	164-18

Mks = Markers; Chrs = chromosomes; Ann Temp.= Annealing Temperature.

APPENDIX B

CARLO SIMULATION (BOOTSTRAP) METHOD TO GENERATE EXPECTED HOMOZYGOTE ALLELE SIZE

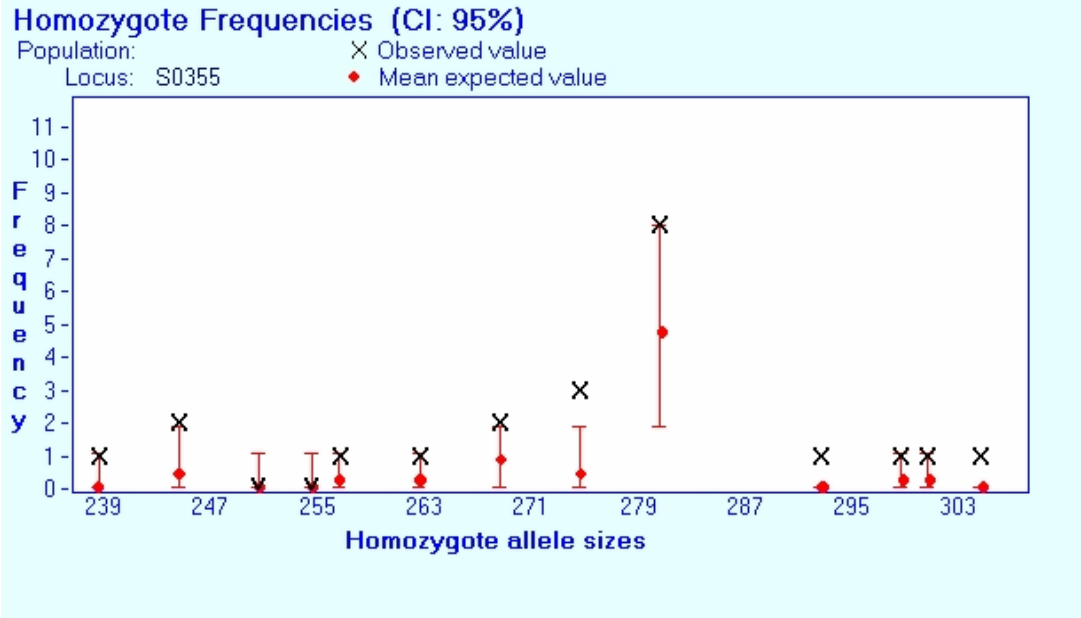

Figure B 1 Carlo simulation (bootstrap) method to generate expected homozygote allele size (uncorrected data). Total expected homozygotes: 7.45, Total observed homozygotes: 22 Combined probability for all classes: $P<0.001$. Null alleles may be present at this locus.

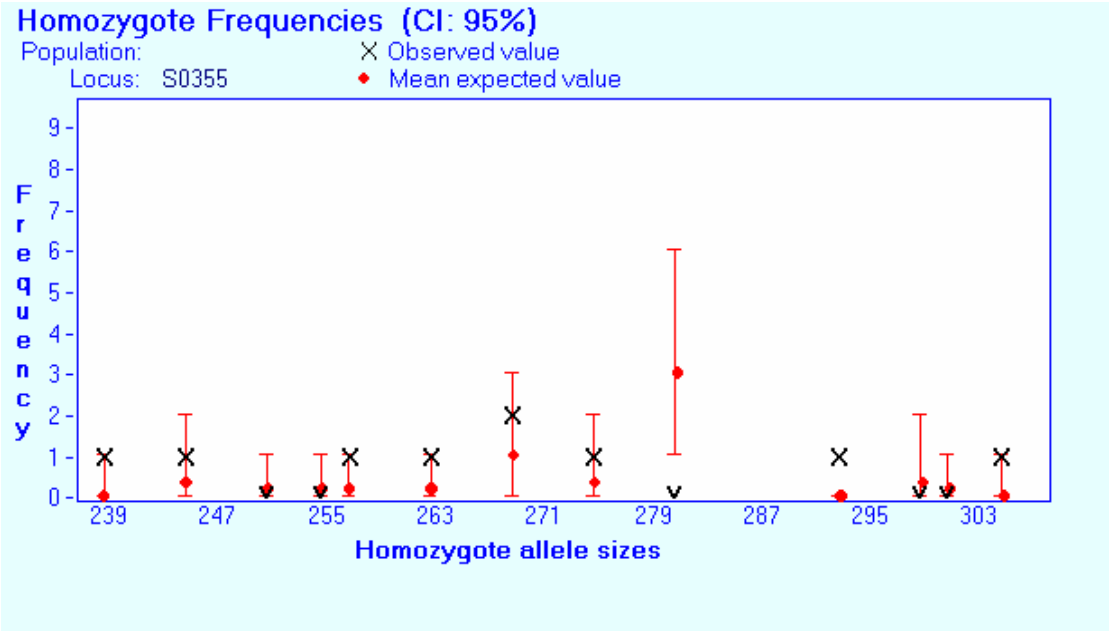

Figure B 2 Carlo simulation (bootstrap) methods to generate expected homozygote allele size (corrected data). Total expected homozygotes:6.17, Total observed homozygotes: 9. Combined probability for all classes: P>0.05. No evidence for presence of null alleles.

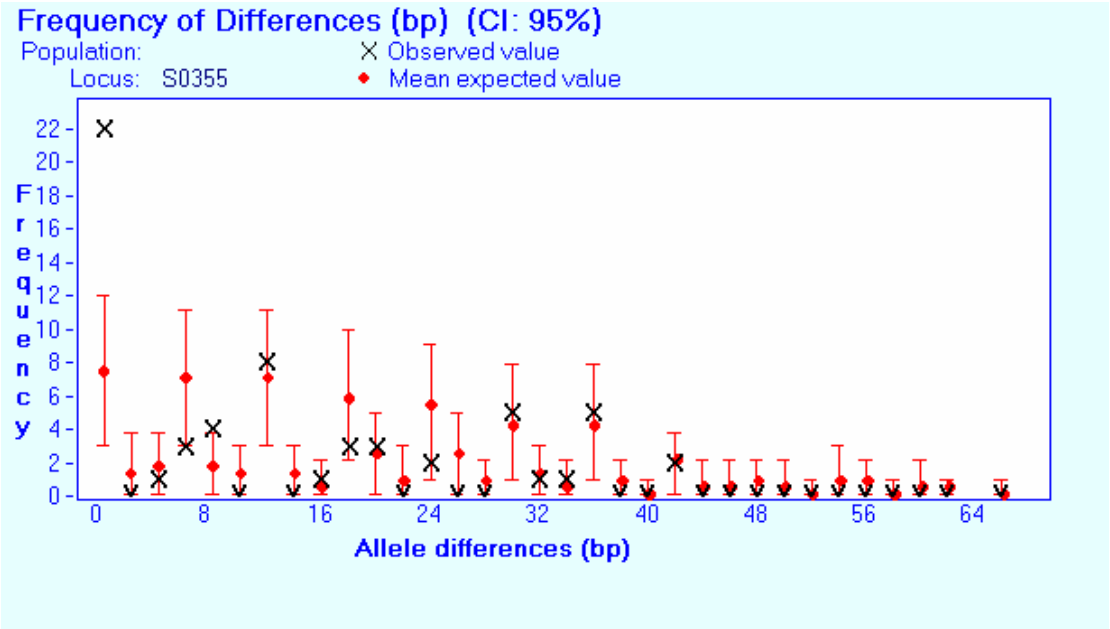

Figure B 3 Carlo simulation (bootstrap) method to generate allele difference (uncorrected data). Combined probability for all classes: P<0.001. (uncorrected data).

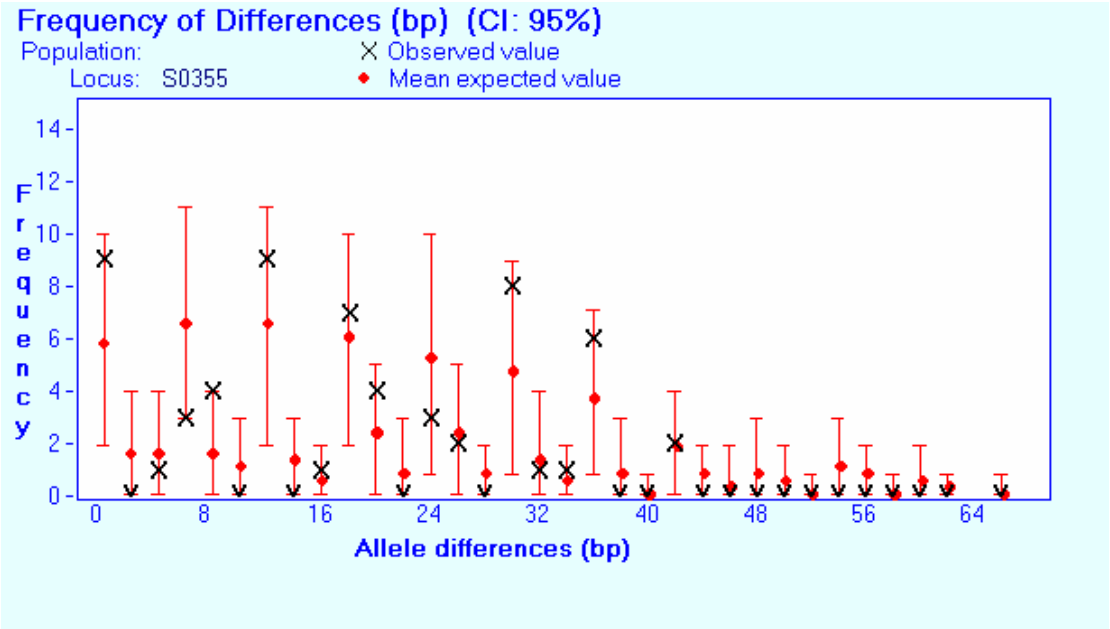

Figure B 3 (Continued) Carlo simulation (bootstrap) method to generate allele difference (corrected data).Combined probability for all classes: P>0.05. (uncorrected data).

APPENDIX C

SEQUENCES OF 1046bp OF *Cyt* b GENE FRAGMENT

IN NINE HAPLOTYPES IN NORTHEAST THAI PIGS

```
H1          1:GACCTCCCAGCCCCCTCAAACATCTCATCATGATGAAACTTCGGTTCCCTCTTAGGCATC 60
H2          1:GACCTCCCAGCCCCCTCAAACATCTCATCATGATGAAACTTCGGTTCCCTCTTAGGCATC 60
H6          1:GACCTCCCAGCCCCCTCAAACATCTCATCATGATGAAACTTCGGTTCCCTCTTAGGCATC 60
H4          1:GACCTCCCAGCCCCCTCAAACATCTCATCATGATGAAACTTCGGTTCCCTCTTAGGCATC 60
H5          1:GACCTCCCAGCCCCCTCAAACATCTCATCATGATGAAACTTCGGTTCCCTCTTAGGCATC 60
H9          1:GACCTCCCAGCCCCCTCAAACATCTCATCATGATGAAACTTCCGTTCCCTCCTAGGCATC 60
H3          1:GACCTCCCAGCCCCCTCAAACATCTCATCATGATGAAACTTCGGTTCCCTCTTAGGCATC 60
H8          1:GACCTCCCAGCCCCCTCAAACATCTCATCATGATGAAACTTCGGTTCCCTCTTAGGCATC 60
H7          1:GACCTCCCAGCCCCCTCAAACATCTCATCATGATGAAACTTCGGTTCCCTCTTAGGCATC 60
              ************************************** ******** ********

H1         61:TGCCTAATCTTGCAAATCCTAACAGGCCTGTTCTTAGCAATACATTACACATCAGACACA 120
H2         61:TGCCTAATCTTGCAAATCCTAACAGGCCTGTTCTTAGCAATACATTACACATCAGACACA 120
H6         61:TGCCTAATCTTGCAAATCCTAACAGGCCTGTTCTTAGCAATACATTACACATCAGACACA 120
H4         61:TGCCTAATCTTGCAAATCCTAACAGGCCTGTTCTTAGCAATACATTACACATCAGACACA 120
H5         61:TGCCTAATCTTGCAAATCCTAACAGGCCTGTTCTTAGCAATACATTACACATCAGACACA 120
H9         61:TGCCTAATCCTGCAAATCCTAACAGGCCTGTTCTTAGCAATACATTACACATCAGACACA 120
H3         61:TGCCTAATCTTGCAAATCCTAACAGGCCTGTTCTTAGCAATACATTACACATCAGACACA 120
H8         61:TGCCTAATCTTGCAAATCCTAACAGGCCTGTTCTTAGCAATACATTACACATCAGACACA 120
H7         61:TGCCTAATCTTGCAAATCCTAACAGGCCTGTTCTTAGCAATACATTACACATCAGACACA 120
              ********* ************************************************

H1        121:ACAACAGCTTTCTCATCAGTTACACACATCTGTCGAGACGTAAATTACGGATGAGTTATT 180
H2        121:ACAACAGCTTTCTCATCAGTTACACACATCTGTCGAGACGTAAATTACGGATGAGTTATT 180
H6        121:ACAACAGCTTTCTCATCAGTTACACACATCTGTCGAGACGTAAATTACGGATGAGTTATT 180
H4        121:ACAACAGCTTTCTCATCAGTTACACACATCTGTCGAGACGTAAATTACGGATGAGTTATT 180
H5        121:ACAACCGCTTTCTCATCAGTTACACACATCTGTCGAGACGTAAATTACGGATGAGTTATT 180
H9        121:ACAACCGCTTTCTCCTCAGTTACCCCCATCTGTCCAGACCTAAATTACGGATGAGTTATT 180
H3        121:ACAACAGCTTTCTCATCAGTTACACACATCTGTCGAGACGTAAATTACGGATGAGTTATT 180
H8        121:ACAACAGCTTTCTCATCAGTTACACACATCTGTCGAGACGCAAATTACGGATGAGTTATT 180
H7        121:ACAACAGCTTTCTCATCAGTTACACACATCTGTCGAGACGTAAATTACGGATGAGTTATT 180
              ***** ******** ******** * ******** ****  ********************

H1        181:CGCTACCTACATGCAAACGGAGCATCCATGTTCTTTATTTGCCTATTCATCCACGTAGGC 240
H2        181:CGCTACCTACATGCAAACGGAGCATCCATGTTCTTTATTTGCCTATTCATCCACGTAGGC 240
H6        181:CGCTACCTACATGCAAACGGAGCATCCATGTTCTTTATTTGCCTATTCATCCACGTAGGC 240
H4        181:CGCTACCTACATGCAAACGGAGCATCCATGTTCTTTATTTGCCTATTCATCCACGTAGGC 240
H5        181:CGCTACCTACATGCAAACGGAGCATCCATGTTCTTTATTTGCCTATTCATCCACGTAGGC 240
H9        181:CGCTACCTACCTGCAAACGGAGCATCCCTGTTCTTTATTTGCCTATTCCTCCACGTAGGC 240
H3        181:CGCTACCTACATGCAAACGGAGCATCCATGTTCTTTATTTGCCTATTCATCCACGTAGGC 240
H8        181:CGCTACCTACATGCAAACGGAGCATCCATGTTCTTTATTTGCCTATTCATCCACGTAGGC 240
H7        181:CGCTACCTACATGCAAACGGAGCATCCATGTTCTTTATTTGCCTATTCATCCACGTAGGC 240
              ********** ********************** ********************** **********
```

```
H1        241:CGAGGCCTATACTACGGATCCTATATATTCCTAGAAACATGAAACATTGGAGTAGTCCTA 300
H2        241:CGAGGCCTATACTACGGATCCTATATATTCCTAGAAACATGAAACATTGGAGTAGTCCTA 300
H6        241:CGAGGCCTATACTACGGATCCTATATATTCCTAGAAACATGAAACATTGGAGTAGTCCTA 300
H4        241:CGAGGCCTATACTACGGATCCTATATATTCCTAGAAACATGAAACATTGGAGTAGTCCTA 300
H5        241:CGAGGCCTATACTACGGATCCTATATATTCCTAGAAACATGAAACATTGGAGTAGTCCTA 300
H9        241:CGAGGCCTATACTACGGATCCTATATATTCCTAGAAACATGAAACATTGGAGTAGTCCTA 300
H3        241:CGAGGCCTATACTACGGATCCTATATATTCCTAGAAACATGAAACATTGGAGTAGTCCTA 300
H8        241:CGAGGCCTATACTACGGATCCTATATATTCCTAGAAACATGAAACATTGGAGTAGTCCTA 300
H7        241:CGAGGCCTATACTACGGATCCTATATATTCCTAGAAACATGAAACATTGGAGTAGTCCTA 300
              ************************************************************

H1        301;CTATTTACCGTTATAGCAACAGCCTTCATAGGCTACGTCCTGCCCTGAGGACAAATATCA 360
H2        301;CTATTTACCGTTATAGCAACAGCCTTCATAGGCTACGTCCTGCCCTGAGGACAAATATCA 360
H6        301;CTATTTACCGTTATAGCAACAGCCTTCATAGGCTACGTCCTGCCCTGAGGACAAATATCA 360
H4        301;CTATTTACCGTTATAGCAACAGCCTTCATAGGCTACGTCCTGCCCTGAGGACAAATATCA 360
H5        301;CTATTTACCGTTATAGCAACAGCCTTCATAGGCTACGTCCTGCCCTGAGGACAAATATCA 360
H9        301;CTATTTACCGTTATAGCAACAGCCTTCATAGGCTACGTCCTGCCCTGAGGACAAATATCA 360
H3        301;CTATTTACCGTTATAGCAACAGCCTTCATAGGCTACGTCCTGCCCTGAGGACAAATATCA 360
H8        301;CTATTTACCGTCATAGCAACAGCCTTCATAGGCTACGTCCTGCCCTGAGGACAAATATCA 360
H7        301;CTATTTACCGTCATAGCAACAGCCTTCATAGGCTACGTCCTGCCCTGAGGACAAATATCA 360
              *********** ************************************************

H1        361:TTCTGAGGAGCTACGGTCATCACAAATCTACTATCAGCTATCCCTTATATCGGAACAGAC 420
H2        361:TTCTGAGGAGCTACGGTCATCACAAATCTACTATCAGCTATCCCTTATATCGGAACAGAC 420
H6        361:TTCTGAGGAGCTACGGTCATCACAAATCTACTATCAGCTATCCCTTATATCGGAACAGAC 420
H4        361:TTCTGAGGAGCTACGGTCATCACAAATCTACTATCAGCTATCCCTTATATCGGAACAGAC 420
H5        361:TTCTGAGGAGCTACGGTCATCACAAATCTACTATCAGCTATCCCTTATATCGGAACAGAC 420
H9        361:TTTTGAGGAGCTACGGTCATCACAAATCTACTATCAGCTATCCCTTATATCGGAACAGAC 420
H3        361:TTCTGAGGAGCTACGGTCATCACAAATCTACTATCAGCTATCCCTTATATCGGAACAGAC 420
H8        361:TTCTGAGGAGCTACGGTCATCACAAATCTACTATCAGCTATCCCTTATATCGGAACAGAC 420
H7        361:TTCTGAGGAGCTACGGTCATCACAAATCTACTATCAGCTATCCCTTATATCGGAACAGAC 420
              ** ************************************************************

H1        421:CTCGTAGAATGAATCTGAGGGGGCTTTTCCGTCGACAAAGCAACCCTCACACGATTCTTC 480
H2        421:CTCGTAGAATGAATCTGAGGGGGCTTTTCCGTCGACAAAGCAACCCTCACACGATTCTTC 480
H6        421:CTCGTAGAATGAATCTGAGGGGGCTTTTCCGTCGACAAAGCAACCCTCACACGATTCTTC 480
H4        421:CTCGTAGAATGAATCTGAGGGGGCTTTTCCGTCGACAAAGCAACCCTCACACGATTCTTC 480
H5        421:CTCGTAGAATGAATCTGAGGGGGCTTTTCCGTCGACAAAGCAACCCTCACACGATTCTTC 480
H9        421:CTCGTAGAATGAATCTGAGGGGGCTTTTCCGTCGACAAAGCAACCCTCACACGATTCTTC 480
H3        421:CTCGTAGAATGAATCTGAGGGGGCTTTTCCGTCGACAAAGCAACCCTCACACGATTCTTC 480
H8        421:CTCGTAGAATGAATCTGAGGGGGCTTTTCCGTCGACAAAGCAACCCTCACACGATTCTTC 480
H7        421:CTCGTAGAATGAATCTGAGGGGGCTTTTCCGTCGACAAAGCAACCCTCACACGATTCTTC 480
              ************************************************************
```

```
H1        481:GCCTTTCACTTTATCCTGCCATTCATCATTACCGCCCTCGCAGCCGTACATCTCCTATTC 540
H2        481:GCCTTTCACTTTATCCTGCCATTCATCATTACCGCCCTCGCAGCCGTACATCTCCTATTC 540
H6        481:GCCTTTCACTTTATCCTGCCATTCATCATTACCGCCCTCGCAGCCGTACATCTCCTATTC 540
H4        481:GCCTTTCACTTTATCCTGCCATTCATCATTACCGCCCTCGCAGCCGTACATCTCCTATTC 540
H5        481:GCCTTTCACTTTATCCTGCCATTCATCATTACCGCCCTCGCAGCCGTACATCTCCTATTC 540
H9        481:GCCTTTCACTTTATCCTGCCATTCATCATTACCGCCCTCGCAGCCGTACATCTCCTATTC 540
H3        481:GCCTTTCACTTTATCCTGCCATTCATCATTACCGCCCTCGCAGCCGTACATCTCCTATTC 540
H8        481:GCCTTTCACTTTATCCTGCCATTCATCATTACCGCCCTCGCAGCGGTACATCTCCTATTC 540
H7        481:GCCTTTCACTTTATCCTGCCATTCATCATTACCGCCCTCGCAGCCGTACATCTCCTATTC 540
              ****************************************** **************

H1        541:CTGCAGGAAACCGGATCCAACAACCCTACCGGAATCTCATCAGACATAGACAAAATTCCA 600
H2        541:CTGCACGAAACCGGATCCAACAACCCTACCGGAATCTCATCAGACATAGACAAAATTCCA 600
H6        541:CTGCACGAAACCGGATCCAACAACCCTACCGGAATCTCATCAGACATAGACAAAATTCCA 600
H4        541:CTGCACGAAACCGGATCCAACAACCCTACCGGAATCTCATCAGACATAGACAAAATTCCA 600
H5        541:CTGCACGAAACCGGATCCAACAACCCTACCGGAATCTCATCAGACATAGACAAAATTCCA 600
H9        541:CTGCACGAAACCGGATCCAACAACCCTACCGGAATCTCATCAGACATAGACAAAATTCCA 600
H3        541:CTGCACGAAACCGGATCCAACAACCCTACCGGAATCTCATCAGACATAGACAAAATTCCA 600
H8        541:CTGCACGAAACGGGATCCAACAACCCTACGGGAATCTCATCAGACATAGACAAAATTCCA 600
H7        541:CTGCACGAAACCGGATCCAACAACCCTACCGGAATCTCATCAGACATAGACAAAATTCCA 600
              ***** ***** **************** ******************************

H1        601:TTTCACCCATACTACACTATTAAAGACATTCTAGGGGCCTTATTTATAATACTAATCCTA 660
H2        601:TTTCACCCATACTACACTATTAAAGACATTCTAGGGGCCTTATTTATAATACTAATCCTA 660
H6        601:TTTCACCCATACTACACTATTAAAGACATTCTAGGGGCCTTATTTATAATACTAATCCTA 660
H4        601:TTTCACCCATACTACACTATTAAAGACATTCTAGGGGCCTTATTTATAATACTAATCCTA 660
H5        601:TTTCACCCATACTACACTATTAAAGACATTCTAGGGGCCTTATTTATAATACTAATCCTA 660
H9        601:TTTCACCCATACTACACTATTAAAGACATTCTAGGGGCCTTATTTATAATACTAATCCTA 660
H3        601:TTTCACCCATACTACACTATTAAAGACATTCTAGGGGCCTTATTTATAATACTAATCCTA 660
H8        601:TTTCACCCATACTACACTATTAAAGACATTTTAGGGGCCTTATTTATAATACTAATCCTA 660
H7        601:TTTCACCCATACTACACTATTAAAGACATTCTAGGGGCCTTATTTATAATACTAATCCTA 660
              ****************************** ******************************

H1        661:CTAATCCTTGTACTATTCTCACCAGACCTACTAGGAGACCCAGACAACTACACCCCAGCA 720
H2        661:CTAATCCTTGTACTATTCTCACCAGACCTACTAGGAGACCCAGACAACTACACCCCAGCA 720
H6        661:CTAATCCTTGTACTATTCTCACCAGACCTACTAGGAGACCCAGACAACTACACCCCAGCA 720
H4        661:CTAATCCTTGTACTATTCTCACCAGACCTACTAGGAGACCCAGACAACTACACCCCAGCA 720
H5        661:CTAATCCTTGTACTATTCTCACCAGACCTACTAGGAGACCCAGACAACTACACCCCAGCA 720
H9        661:CTAATCCTTGTACTATTCTCACCAGACCTACTAGGAGACCCAGACAACTACACCCCAGCA 720
H3        661:CTAATCCTTGTACTATTCTCACCAGACCTACTAGGAGACCCAGACAACTACACCCCAGCA 720
H8        661:CTAATCCTTGTACTATTCTCACCAGACCTATTAGGAGACCCAGACAACTACACCCCAGCA 720
H7        661:CTAATCCTTGTACTATTCTCACCAGACCTACTAGGAGACCCAGACAACTACACCCCAGCA 720
              ****************************** ******************************
```

```
H1        721:AACCCACTAAACACCCCACCCCATATTAAACCAGAATGATATTTCTTATTCGCCTACGCT 780
H2        721:AACCCACTAAACACCCCACCCCATATTAAACCAGAATGATATTTCTTATTCGCCTACGCT 780
H6        721:AACCCACTAAACACCCCACCCCATATTAAACCAGAATGATATTTCTTATTCGCCTACGCT 780
H4        721:AACCCACTAAACACCCCACCCCATATTAAACCAGAATGATATTTCTTATTCGCCTACGCT 780
H5        721:AACCCACTAAACACCCCACCCCATATTAAACCAGAATGATATTTCTTATTCGCCTACGCT 780
H9        721:AACCCACTAAACACCCCACCCCATATTAAACCAGAATGATATTTCTTATTCGCCTACGCT 780
H3        721:AACCCACTAAACACCCCACCCCATATTAAACCAGAATGATATTTCTTATTCGCCTACGCT 780
H8        721:AACCCACTAAACACCCCACCCCATATTAAACCAGAAGGATATTTTTTATTCGCTTACGCT 780
H7        721:AACCCACTAAACACCCCCCCCCATATTAAACCAGAATGATATTTCTTATTCGCCTACGCT 780
              ********** ********** ******* ******** ******

H1        781:ATCCTACGTTCAATTCCTAATAAACTAGGTGGAGTGCTAGCTCTAATAGCCTCCATCCTA 840
H2        781:ATCCTACGTTCAATTCCTAATAAACTAGGTGGAGTGCTAGCTCTAATAGCCTCCATCCTA 840
H6        781:ATCCTACGTTCAATTCCTAATAAACTAGGTGGAGTGCTAGCTCTAATAGCCTCCATCCTA 840
H4        781:ATCCTACGTTCAATTCCTAATAAACTAGGTGGAGTGCTAGCTCTAGTAGCCTCCATCCTA 840
H5        781:ATCCTACGTTCAATTCCTAATAAACTAGGTGGAGTGCTAGCTCTAGTAGCCTCCATCCTA 840
H9        781:ATCCTACGTTCAATTCCTAATAAACTAGGTGGAGTGCTAGCTCTAGTAGCCTCCATCCTA 840
H3        781:ATCCTACGTTCAATTCCTAATAAACTAGGTGGAGTGCTAGCTCTAGTAGCCTCCATCCTA 840
H8        781:ATCTTACGTTCAATTCTTAATAAACTAGGGGGAGTGCTAGCTCTAGTAGCCTCCATCCTA 840
H7        781:ATCGTGCGTTCAATTCATAATAAACTGGGGGGAGTGCTAGCTCTAGTAGCCTCCATCTTA 840
              *** * ********** ********* ** ********** ********** **

H1        841:ATCCTAATTTTAATGCCCATACTACACACATCCAAACAACGAAGCATAATATTTCGACCA 900
H2        841:ATCCTAATTTTAATGCCCATACTACACACATCCAAACAACGAAGCATAATATTTCGACCA 900
H6        841:ATCCTAATTTTAATGCCCATACTACACACATCCAAACAACGAAGCATAATATTTCGACCA 900
H4        841:ATCCTAATTTTAATGCCCATACTACACACATCCAAACAACGAAGCATAATATTTCGACCA 900
H5        841:ATCCTAATTTTAATGCCCATACTACACACATCCAAACAACGAAGCATAATATTTCGACCA 900
H9        841:ATCCTAATTTTAATGCCCATACTACACACATCCAAACAACGAAGCATAATATTTCGACCA 900
H3        841:ATCCTAATTTTAATGCCCATACTACACACATCCAAACAACGAAGCATAATATTTCGACCA 900
H8        841:ATCCTAATTTTAATGCCCATACTACACACATCCAAACAACGAAGCATAATATTTCGACCA 900
H7        841:ATCCTAATTTTAATGCCCATAATACACACATCCAAACAAGGAAGCATAATATTTCGACCA 900
              ********************** ********** **********

H1        901:CTAAGTCAATGCCTATTCTGAATACTAGTAGCAGACCTCATTACACTAACATGAATTGGA 960
H2        901:CTAAGTCAATGCCTATTCTGAATACTAGTAGCAGACCTCATTACACTAACATGAATTGGA 960
H6        901:CTAAGTCAATACCTATTTTGAATACTAGTAGCAGACCTCATTACACTAACATGAATTGGA 960
H4        901:CTAAGTCAATGCCTATTGTGAATACTAGTAGCAGACCTCATTACACTAACATGAATTGGA 960
H5        901:CTAAGTCAATGCCTATTCTGAATACTAGTAGCAGACCTCATTACACTAACATGAATTGGA 960
H9        901:CTAAGTCAATGCCTATTCTGAATACTAGTAGCAGACCTCATTACACTAACATGAATTGGA 960
H3        901:CTAAGTCAATGCCTATTCTGAATACTAGTAGCAGACCTCATTACACTAACATGAATTGGA 960
H8        901:CTAAGTCAATGCCTATTCTGAATACTAGTAGCAGACCTCATTACACTAACATGAATTGGA 960
H7        901:CTAAGTCAATGCTTATTCTGAATAGTAGTAGCAGACCTCATTCCACTAACATGAATGGGA 960
              ********** * **** ****** ********** ********** ***
```

```
H1        961:GGACAACCCGTAGAACACCCATTCATCATCATCGGCCAACTAGCCTCCATCTTATATTTC 1020
H2        961:GGACAACCCGTAGAACACCCATTCATCATCATCGGCCAACTAGCCTCCATCTTATATTTC 1020
H6        961:GGACAACCCGTAGAACACCCATTCATCATCATCGGCCAACTAGCCTCCATCTTAAATTTC 1020
H4        961:GGACAACCCGTAGAACACCCATTCATCATCATCGGCCAACTAGCCTCCATCTTATATTTC 1020
H5        961:GGACAACCCGTAGAACACCCATTCATCATCATCGGCCAACTAGCCTCCATCTTATATTTC 1020
H9        961:GGACAACCCGTAGAACACCCATTCATCATCATCGGCCAACTAGCCTCCATCTTATATTTC 1020
H3        961:GGACAACCCGTAGAACACCCATTCATCATCATCGGCCAACTAGCCTCCATCTTATATTTC 1020
H8        961:GGACAACCCGTAGAACACCCATTCATCATCATCGGCCAACTAGCCTCCATCTTATATTTC 1020
H7        961:GGACAACCCGTAGAACACCCATTCATCATCATCGGCCAAGTAGCCTCCATCTAATATTTC 1020
              ************************************ ************ * *****

H1       1021:CTAATCATTCTAGTATTGATACCAAT 1046
H2       1021:CTAATCATTCTAGTATTGATACCAAT 1046
H6       1021:CTAATCATTCTAGTATTGATACCAAT 1046
H4       1021:CTAATCATTCTAGTATTGATACCAAT 1046
H5       1021:CTAATCATTCTAGTATTGATACCAAT 1046
H9       1021:CTAATCATTCTAGTATTGATACCAAT 1046
H3       1021:CTAATCATTCTAGTATTGATACCAAT 1046
H8       1021:CTAATCATTCTAGTATTGATACCAAT 1046
H7       1021:CTAATCATTCTAGTATTGATACCAAT 1046
              *************************
```

Figure C 1 Sequences of 1046bp of *Cyt* B gene fragment in nine haplotypes in Northeast Thai pig population

APPENDIX D

SEQUENCES OF 1046bp OF *Cyt* b GENE FRAGMENT IN FIFTEEN HAPLOTYPES FROM EXTPOIC PIG BREEDS

```
HWZS        1:GACCTCCCAGCCCCCTCAAACATCTCATCATGATGAAACTTCGGTTCCCTCTTAGGCATC 60
HLW         1:GACCTCCCAGCCCCCTCAAACATCTCATCATGATGAAACTTCGGTTCCCTCTTAGGCATC 60
HJP2        1:GACCTCCCAGCCCCCTCAAACATCTCATCATGATGAAACTTCGGTTCCCTCTTAGGCATC 60
HVN         1:GACCTCCCAGCCCCCTCAAACATCTCATCATGATGAAACTTCGGTTCCCTCTTAGGCATC 60
HKR1        1:GACCTCCCAGCCCCCTCAAACATCTCATCATGATGAAACTTCGGTTCCCTCTTAGGCATC 60
HRC         1:GACCTCCCAGCCCCCTCAAACATCTCATCATGATGAAACTTCGGTTCCCTCTTAGGCATC 60
HX          1:GACCTCCCAGCCCCCTCAAACATCTCATCATGATGAAACTTCGGTTCCCTCTTAGGCATC 60
HJH         1:GACCTCCCAGCCCCCTCAAACATCTCATCATGATGAAACTTCGGTTCCCTCTTAGGCATC 60
HMS         1:GACCTCCCAGCCCCCTCAAACATCTCATCATGATGAAACTTCGGTTCCCTCTTAGGCATC 60
HKR2        1:GACCTCCCAGCCCCCTCAAACATCTCATCATGATGAAACTTCGGTTCCCTCTTAGGCATC 60
HJP1        1:GACCTCCCAGCCCCCTCAAACATCTCATCATGATGAAACTTCGGTTCCCTCTTAGGCATC 60
HYN         1:GACCTCCCAGCCCCCTCAAACATCTCATCATGATGAAACTTCGGTTCCCTCTTAGGCATC 60
HEW1        1:GACCTCCCAGCCCCCTCAAACATCCCATCATGATGAAACTTCGGTTCCCTCTTAGGCATC 60
HEW2        1:GACCTCCCAGCCCCCTCAAACATCCCATCATGATGAAACTTCGGTTCCCTCTTAGGCATC 60
HDU         1:GACCTCCCAGCCCCCTCAAACATCTCATCATGATGAAACTTCGGTTCCCTCTTAGGCATC 60
              ********************** **********************************

HWZS       61:TGCCTAATCTTGCAAATCCTAACAGGCCTGTTCTTAGCAATACATTACACATCAGACACA 120
HLW        61:TGCCTAATCTTGCAAATCCTAACAGGCCTGTTCTTAGCAATACATTACACATCAGACACA 120
HJP2       61:TGCCTAATCTTGCAAATCCTAACAGGCCTGTTCTTAGCAATACATTACACATCAGACACA 120
HVN        61:TGCCTAATCTTGCAAATCCTAACAGGCCTGTTCTTAGCAATACATTACACATCAGACACA 120
HKR1       61:TGCCTAATCTTGCAAATCCTAACAGGCCTGTTCTTAGCAATACATTACACATCAGACACA 120
HRC        61:TGCCTAATCTTGCAAATCCTAACAGGCCTGTTCTTAGCAATACATTACACATCAGACACA 120
HX         61:TGCCTAATCTTGCAAATCCTAACAGGCCTGTTCTTAGCAATACATTACACATCAGACACA 120
HJH        61:TGCCTAATCTTGCAAATCCTAACAGGCCTGTTCTTAGCAATACATTACACATCAGACACA 120
HMS        61:TGCCTAATCTTGCAAATCCTAACAGGCCTGTTCTTAGCAATACATTACACATCAGACACA 120
HKR2       61:TGCCTAATCTTGCAAATCCTAACAGGCCTGTTCTTAGCAATACATTACACATCAGACACA 120
HJP1       61:TGCCTAATCTTGCAAATCCTAACAGGCCTGTTCTTAGCAATACATTACACATCAGACACA 120
HYN        61:TGCCTAATCTTGCAAATCCTAACAGGCCTGTTCTTAGCAATACATTACACATCAGACACA 120
HEW1       61:TGCCTAATCTTGCAAATCCTAACAGGCCTATTCTTAGCAATACATTACACATCAGACACA 120
HEW2       61:TGCCTAATCTTGCAAATCCTAACAGGCCTATTCTTAGCAATACATTACACATCAGACACA 120
HDU        61:TGCCTAATCTTGCAAATCCTAACAGGCCTGTTCTTAGCAATACATTACACATCAGACACA 120
             ************************** ***************************
```

```
HWZS      121:ACAACAGCTTTCTCATCAGTTACACACATCTGTCGAGACGTAAATTACGGATGAGTTATT 180
HLW       121:ACAACAGCTTTCTCATCAGTTACACACATCTGTCGAGATGTAAATTACGGATGAGTTATT 180
HJP2      121:ACAACAGCTTTCTCATCAGTTACACACATCTGTCGAGACGTAAATTACGGATGAGTTATT 180
HVN       121:ACAACAGCTTTCTCATCAGTTACACACATCTGTCGAGACGTAAATTACGGATGAGTTATT 180
HKR1      121:ACAACAGCTTTCTCATCAGTTACACACATCTGTCGAGACGTAAATTACGGATGAGTTATT 180
HRC       121:ACAACAGCTTTCTCATCAGTTACACACATCTGTCGAGACGTAAATTACGGATGAGTTATT 180
HX        121:ACAACAGCTTTCTCATCAGTTACACACATCTGTCGAGACGTAAATTACGGATGAGTTATT 180
HJH       121:ACAACAGCTTTCTCATCAGTTACACACATCTGTCGAGACGTAAATTACGGATGAGTTATT 180
HMS       121:ACAACAGCTTTCTCATCAGTTACACACATCTGTCGAGACGTAAATTACGGATGAGTTATT 180
HKR2      121:ACAACAGCTTTCTCATCAGTTACACACATCTGTCGAGACGTAAATTACGGATGAGTTATT 180
HJP1      121:ACAACAGCTTTCTCATCAGTTACACACATCTGTCGAGACGTAAATTACGGATGAGTTATT 180
HYN       121:ACAACAGCTTTCTCATCAGTTACACACATCTGTCGAGACGTAAACTACGGATGAGTTATT 180
HEW1      121:ACAACAGCTTTCTCATCAGTTACACACATCTGTCGAGACGTAAATTACGGATGAGTTATT 180
HEW2      121:ACAACAGCTTTCTCATCAGTTACACACATCTGTCGAGACGTAAATTACGGATGAGTTATT 180
HDU       121:ACAACAGCTTTCTCATCAGTTACACACATTTGTCGAGACGTAAATTACGGATGAGTTATT 180
                ************************** ******** ***** **************

HWZS      181:CGCTACCTACATGCAAACGGAGCATCCATGTTCTTTATTTGCCTATTCATCCACGTAGGC 240
HLW       181:CGCTACCTACATGCAAACGGAGCATCCATGTTCTTTATTTGCCTATTCATCCACGTAGGC 240
HJP2      181:CGCTACCTACATGCAAACGGAGCATCCATGTTCTTTATTTGCCTATTCATCCACGTAGGC 240
HVN       181:CGCTACCTACATGCAAACGGAGCATCCATGTTCTTTATTTGCCTATTCATCCACGTAGGC 240
HKR1      181:CGCTACCTACATGCAAACGGAGCATCCATGTTCTTTATTTGCCTATTCATCCACGTAGGC 240
HRC       181:CGCTACCTACATGCAAACGGAGCATCCATGTTCTTTATTTGCCTATTCATCCACGTAGGC 240
HX        181:CGCTACCTACATGCAAACGGAGCATCCATGTTCTTTATTTGCCTATTCATCCACGTAGGC 240
HJH       181:CGCTACCTACATGCAAACGGAGCATCCATGTTCTTTATTTGCCTATTCATCCACGTAGGC 240
HMS       181:CGCTACCTACATGCAAACGGAGCATCCATGTTCTTTATTTGCCTATTCATCCACGTAGGC 240
HKR2      181:CGCTACCTACATGCAAACGGAGCATCCATGTTCTTTATTTGCCTATTCATCCACGTAGGC 240
HJP1      181:CGCTACCTACATGCAAACGGAGCATCCATGTTCTTTATTTGCCTATTCATCCACGTAGGC 240
HYN       181:CGCTACCTACATGCAAACGGAGCATCCATATTCTTTATTTGCCTATTCATCCACGTAGGC 240
HEW1      181:CGCTACCTACATGCAAACGGAGCATCCATATTCTTTATTTGCCTATTCATCCACGTAGGC 240
HEW2      181:CGCTACCTACATGCAAACGGAGCATCCATATTCTTTATTTGCCTATTCATCCACGTAGGC 240
HDU       181:CGCTATCTACATGCAAACGGAGCATCCATATTCTTTATTTGCCTATTCATCCACGTAGGC 240
                ***** ********************** ****************************

HWZS      241:CGAGGCCTATACTACGGATCCTATATATTCCTAGAAACATGAAACATTGGAGTAGTCCTA 300
HLW       241:CGAGGCCTATACTACGGATCCTATATATTCCTAGAAACATGAAACATTGGAGTAGTCCTA 300
HJP2      241:CGAGGCCTATACTACGGATCCTATATATTCCTAGAAACATGAAACATTGGAGTAGTCCTA 300
HVN       241:CGAGGCCTATACTACGGATCCTATATATTCCTAGAAACATGAAACATTGGAGTAGTCCTA 300
HKR1      241:CGAGGCCTATACTACGGATCCTATATATTCCTAGAAACATGAAACATTGGAGTAGTCCTA 300
HRC       241:CGAGGCCTATACTACGGATCCTATATATTCCTAGAAACATGAAACATTGGAGTAGTCCTA 300
HX        241:CGAGGCCTATACTACGGATCCTATATATTCCTAGAAACATGAAACATTGGAGTAGTCCTA 300
HJH       241:CGAGGCCTATACTACGGATCCTATATATTCCTAGAAACATGAAACATTGGAGTAGTCCTA 300
HMS       241:CGAGGCCTATACTACGGATCCTATATATTCCTAGAAACATGAAACATTGGAGTAGTCCTA 300
HKR2      241:CGAGGTCTATACTACGGATCCTATATATTCCTAGAAACATGAAACATTGGAGTAGTCCTA 300
HJP1      241:CGAGGTCTATACTACGGATCCTATATATTCCTAGAAACATGAAACATTGGAGTAGTCCTA 300
HYN       241:CGAGGTCTATACTACGGATCCTATATATTCCTAAAAACATGAAACATTGGAGTAGTCCTA 300
HEW1      241:CGAGGTCTATACTACGGATCCTATATATTCCTAGAAACATGAAACATTGGAGTAGTCCTA 300
HEW2      241:CGAGGTCTATACTACGGATCCTATATATTCCTAGAAACATGAAACATTGGAGTAGTCCTA 300
HDU       241:CGAGGTCTATACTACGGATCCTATATATTCCTAGAAACATGAAACATTGGAGTAGTCCTA 300
                ***** ************************* *************************
```

```
HWZS      301:CTATTTACCGTTATAGCAACAGCCTTCATAGGCTACGTCCTGCCCTGAGGACAAATATCA 360
HLW       301:CTATTTACCGTTATAGCAACAGCCTTCATAGGCTACGTCCTGCCCTGAGGACAAATATCA 360
HJP2      301:CTATTTACCGTTATAGCAACAGCCTTCATAGGCTACGTCCTGCCCTGAGGACAAATATCA 360
HVN       301:CTATTTACCGTTATAGCAACAGCCTTCATAGGCTACGTCCTGCCCTGAGGACAAATATCA 360
HKR1      301:CTATTTACCGTTATAGCAACAGCCTTCATAGGCTACGTCCTGCCCTGAGGACAAATATCA 360
HRC       301:CTATTTACCGTTATAGCAACAGCCTTCATAGGCTACGTCCTGCCCTGAGGACAAATATCA 360
HX        301:CTATTTACCGTTATAGCAACAGCCTTCATAGGCTACGTCCTGCCCTGAGGACAAATATCA 360
HJH       301:CTATTTACCGTTATAGCAACAGCCTTCATAGGCTACGTCCTGCCCTGAGGACAAATATCA 360
HMS       301:CTATTTACCGTTATAGCAACAGCCTTCATAGGCTACGTCCTGCCCTGAGGACAAATATCA 360
HKR2      301:CTATTTACCGTTATAGCAACAGCCTTCATAGGCTACGTCCTGCCCTGAGGACAAATATCA 360
HJP1      301:CTATTTACCGTTATAGCAACAGCCTTCATAGGCTACGTCCTGCCCTGAGGACAAATATCA 360
HYN       301:CTATTTACCGTTATAGCAACAGCCTTCATAGGCTACGTCCTGCCCTGAGGACAAATATCA 360
HEW1      301:CTATTTACCGTTATAACAACAGCCTTCATAGGCTACATCCTGCCCTGAGGACAAATATCA 360
HEW2      301:CTATTTACCGTTATAACAACAGCCTTCATAGGCTACATCCTGCCCTGAGGACAAATATCA 360
HDU       301:CTATTTACCGTTATAGCAACAGCCTTCATAGGCTACGTCCTGCCCTGAGGACAAATATCA 360
                ********** ********** **********

HWZS      361:TTCTGAGGAGCTACAGTCATCACAAATCTACTATCAGCTATCCCTTATATTGGAACAGAC 420
HLW       361:TTCTGAGGAGCTACAGTCATCACAAATCTACTATCAGCTATCCCTTATATCGGAACAGAC 420
HJP2      361:TTCTGAGGAGCTACGGTCATCACAAATCTACTATCAGCTATCCCTTATATCGGAACAGAC 420
HVN       361:TTCTGAGGAGCTACGGTCATCACAAATCTACTATCAGCTATCCCTTATATCGGAACAGAC 420
HKR1      361:TTCTGAGGAGCTACGGTCATCACAAATCTACTATCAGCTATCCCTTATATCGGAACAGAC 420
HRC       361:TTCTGAGGAGCTACGGTCATCACAAATCTACTATCAGCTATCCCTTATATCGGAACAGAC 420
HX        361:TTCTGAGGAGCTACGGTCATCACAAATCTACTATCAGCTATCCCTTATATCGGAACAGAC 420
HJH       361:TTCTGAGGAGCTACGGTCATCACAAATCTACTATCAGCTATCCCTTATATCGGAACAGAC 420
HMS       361:TTCTGAGGAGCTACGGTCATCACAAATCTACTATCAGCTATCCCTTATATCGGAACAGAC 420
HKR2      361:TTCTGAGGAGCTACGGTCATCACAAATCTACTATCAGCTATCCCTTATATCGGGACAGAC 420
HJP1      361:TTCTGAGGAGCTACGGTCATCACAAATCTACTATCAGCTATCCCTTATATCGGAACAGAC 420
HYN       361:TTCTGAGGAGCTACGGTCATCACAAACCTACTATCAGCTATCCCTTATATCGGAACAGAC 420
HEW1      361:TTCTGAGGAGCTACGGTCATCACAAATCTACTATCAGCTATCCCTTATATCGGAACAGAC 420
HEW2      361:TTCTGAGGAGCTACGGTCATCACAAATCTACTATCAGCTATCCCTTATATCGGAACAGAC 420
HDU       361:TTCTGAGGAGCTACGGTCATCACAAATCTACTATCAGCTATCCCTTATATCGGAACAGAC 420
                ********** ********** ********************** ** ******

HWZS      421:CTCGTAGAATGAATCTGAGGGGGCTTTTCCGTCGACAAAGCAACCCTCACACGATTCTTC 480
HLW       421:CTCGTAGAATGAATCTGAGGGGGCTTTTCCGTCGACAAAGCAACCCTCACACGATTCTTC 480
HJP2      421:CTCGTAGAATGAATCTGAGGGGGCTTTTCCGTCGACAAAGCAACCCTCACACGATTCTTC 480
HVN       421:CTCGTAGAATGAATCTGAGGGGGCTTTTCCGTCGACAAAGCAACCCTCACACGATTCTTC 480
HKR1      421:CTCGTAGAATGAATCTGAGGGGGCTTTTCCGTCGACAAAGCAACCCTCACACGATTCTTC 480
HRC       421:CTCGTAGAATGAATCTGAGGGGGCTTTTCCGTCGACAAAGCAACCCTCACACGATTCTTC 480
HX        421:CTCGTAGAATGAATCTGAGGGGGCTTTTCCGTCGACAAAGCAACCCTCACACGATTCTTC 480
HJH       421:CTCGTAGAATGAATCTGAGGGGGCTTTTCCGTCGACAAAGCAACCCTCACACGATTCTTC 480
HMS       421:CTCGTAGAATGAATCTGAGGGGGCTTTTCCGTCGACAAAGCAACCCTCACACGATTCTTC 480
HKR2      421:CTCGTAGAATGAATCTGAGGGGGCTTTTCCGTCGACAAAGCAACCCTCACACGATTCTTC 480
HJP1      421:CTCGTAGAATGAATCTGAGGGGGCTTTTCCGTCGACAAAGCAACCCTCACACGATTCTTC 480
HYN       421:CTCGTAGAATGAATCTGAGGGGGCTTTTCCGTCGACAAAGCAACCCTCACACGATTCTTC 480
HEW1      421:CTCGTAGAATGAATCTGAGGGGGCTTTTCCGTCGACAAAGCAACCCTCACACGATTCTTC 480
HEW2      421:CTCGTAGAATGAATCTGAGGGGGCTTTTCCGTCGACAAAGCAACCCTCACACGATTCTTC 480
HDU       421:CTCGTAGAATGAATCTGAGGGGGCTTTTCCGTCGACAAAGCAACCCTCACACGATTCTTC 480
                ************************************************************
```

```
HWZS      481:GCCTTTCACTTTATCCTGCCATTCATCATTACCGCCCTCGCAGCCGTACATCTCCTATTC 540
HLW       481:GCCTTCCACTTTATCCTGCCATTCATCATTACCGCCCTCGCAGCCGTACATCTCCTATTC 540
HJP2      481:GCCTTTCACTTTATCCTGCCATTCATCATTACCGCCCTCGCAGCCGTACATCTCCTATTC 540
HVN       481:GCCTTTCACTTTATCCTGCCATTCATCATTACCGCCCTCGCAGCCGTACATCTCCTATTC 540
HKR1      481:GCCTTTCACTTTATCCTGCCATTCATCATTACCGCCCTCGCAGCCGTACATCTCCTATTC 540
HRC       481:GCCTTTCACTTTATCCTGCCATTCATCATTACCGCCCTCGCAGCCGTACATCTCCTATTC 540
HX        481:GCCTTTCACTTTATCCTGCCATTCATCATTACCGCCCTCGCAGCCGTACATCTCCTATTC 540
HJH       481:GCCTTTCACTTTATCCTGCCATTCATCATTACCGCCCTCGCAGCCGTACATCTCCTATTC 540
HMS       481:GCCTTTCACTTTATCCTGCCATTCATCATTACCGCCCTCGCAGCCGTACATCTCCTATTC 540
HKR2      481:GCCCTTCACTTTATCCTGCCATTCATCATTACCGCCCTCGCAGCCGTACATCTCCTATTC 540
HJP1      481:GCCTTTCACTTTATCCTGCCATTCATCATTACCGCCCTCGCAGCCGTACATCTCCTATTC 540
HYN       481:GCCTTTCACTTTATCCTGCCATTCATCATTACCGCCCTCGCAGCCGTACATCTCCTATTC 540
HEW1      481:GCCTTTCACTTTATCCTGCCATTCATCATTACCGCCCTCGCAGCCGTACATCTCCTATTC 540
HEW2      481:GCCTTTCACTTTATCCTGCCATTCATCATTACCGCCCTCGCAGCCGTACATCTCCTATTC 540
HDU       481:GCCTTTCACTTTATCCTGCCATTCATCATTACCGCCCTCGCAGCCGTACATCTCCTATTC 540
               *** * **************************************************

HWZS      541:CTGCACGAAACCGGATCCAACAACCCTACCGGAATCTCATCAGACATAGACAAAATTCCA 600
HLW       541:CTGCACGAAACCGGATCCAACAACCCTACCGGAATCTCATCAGACATAGACAAAATTCCA 600
HJP2      541:CTGCACGAAACCGGATCCAACAACCCTACCGGAATCTCATCAGACATAGACAAAATTCCA 600
HVN       541:CTGCACGAAACCGGATCCAACAACCCTACCGGAATCTCATCAGACATAGACAAAATTCCA 600
HKR1      541:CTGCACGAAACCGGATCCAACAACCCTACCGGAATCTCATCAGACATAGACAAAATTCCA 600
HRC       541:CTGCACGAAACCGGATCCAACAACCCTACCGGAATCTCATCAGACATAGACAAAATTCCA 600
HX        541:CTGCACGAAACCGGATCCAACAACCCTACCGGAATCTCATCAGACATAGACAAAATTCCA 600
HJH       541:CTGCACGAAACCGGATCCAACAACCCTACCGGAATCTCATCAGACATAGACAAAATTCCA 600
HMS       541:CTGCACGAAACCGGATCCAACAACCCTACCGGAATCTCATCAGACATAGACAAAATTCCA 600
HKR2      541:CTGCACGGAACCGGATCCAACAACCCTACCGGAATCTCATGAGACATAGACGAAATTCCA 600
HJP1      541:CTGCACGAAACCGGATCCAACAACCCTACCGGAATCTCATCAGACATAGACAAAATTCCA 600
HYN       541:CTGCACGAAACCGGATCCAATAACCCTACCGGAATCTCATCAGACATAGACAAAATTCCA 600
HEW1      541:CTGCACGAAACCGGATCCAATAACCCTACCGGAATCTCATCAGACATAGACAAAATTCCA 600
HEW2      541:CTGCACGAAACCGGATCCAATAACCCTACCGGAATCTCATCAGACATAGACAAAATTCCA 600
HDU       541:CTGCACGAAACCGGATCCAACAACCCTACCGGAATCTCATCAGACATAGACAAAATTCCA 600
               ******* *********** ****************** ********** ********

HWZS      601:TTTCACCCATACTACACTATTAAAGACATTCTAGGGGCCTTATTTATAATACTAATCCTA 660
HLW       601:TTTCACCCATACTACACTATTAAAGACATTCTAGGGGCCTTATTTATAATACTAATCCTA 660
HJP2      601:TTTCACCCATACTACACTATTAAAGACATTCTAGGGGCCTTATTTATAATACTAATCCTA 660
HVN       601:TTTCACCCATACTACACTATTAAAGACATTCTAGGGGCCTTATTTATAATACTAATCCTA 660
HKR1      601:TTTCACCCATACTACACTATTAAAGACATTCTAGGGGCCTTATTTATAATACTAATCCTA 660
HRC       601:TTTCACCCATACTACACTATTAAAGACATTCTAGGGGCCTTATTTATAATACTAATCCTA 660
HX        601:TTTCACCCATACTACACTATTAAAGACATTCTAGGGGCCTTATTTATAATACTAATCCTA 660
HJH       601:TTTCACCCATACTACACTATTAAAGACATTCTAGGGGCCTTATTTATAATACTAATCCTA 660
HMS       601:TTTCACCCATACTACACTATTAAAGACATTCTAGGGGCCTTATTTATAATACTAATCCTA 660
HKR2      601:TTTCACCCATACTACACTATTAAAGACATTCTAGGGGCCGTATTTATAATACTAATCCTA 660
HJP1      601:TTTCACCCATACTACACTATTAAAGACATTCTAGGGGCCTTATTTATAATACTAATCCTA 660
HYN       601:TTTCACCCATACTACACTATTAAAGACATTCTAGGAGCCTTATTTATAATACTAATCCTA 660
HEW1      601:TTTCACCCATACTACACTATTAAAGACATTCTAGGAACCTTATTTATAATACTAATCCTA 660
HEW2      601:TTTCACCCATACTACACTATTAAAGACATTCTAGGAACCTTATTTATAATACTAATCCTA 660
HDU       601:TTTCACCCATACTACACTATTAAAGACATTCTAGGAGCCTTATTTATAATACTAATCCTA 660
               **********************************  ** ******************
```

```
HWZS      661:CTAATCCTTGTACTATTCTCACCAGACCTACTAGGAGACCCAGACAACTACACCCCAGCA 720
HLW       661:CTAATCCTTGTACTATTCTCACCAGACCTACTAGGAGACCCAGACAACTACACCCCAGCA 720
HJP2      661:CTAATCCTTGTACTATTCTCACCAGACCTACTAGGAGACCCAGACAACTACACCCCAGCA 720
HVN       661:CTAATCCTTGTACTATTCTCACCAGACCTACTAGGAGACCCAGACAACTACACCCCAGCA 720
HKR1      661:CTAATCCTTGTACTATTCTCACCAGACCTACTAGGAGACCCAGACAACTACACCCCAGCA 720
HRC       661:CTAATCCTTGTACTATTCTCACCAGACCTACTAGGAGACCCAGACAACTACACCCCAGCA 720
HX        661:CTAATCCTTGTACTATTCTCACCAGACCTACTAGGAGACCCAGACAACTACACCCCAGCA 720
HJH       661:CTAATCCTTGTACTATTCTCACCAGACCTACTAGGAGACCCAGACAACTACACCCCAGCA 720
HMS       661:CTAATCCTTGTACTATTCTCACCAGACCTACTAGGAGACCCAGACAACTACACCCCAGCA 720
HKR2      661:CTAATCCTTGTACTATTCTCACCAGACCTACTAGGAGACCCAGACAACTACACCCCAGCA 720
HJP1      661:CTAATCCTTGTACTATTCTCACCAGACCTACTAGGAGACCCAGACAACTACACCCCAGCA 720
HYN       661:CTAATCCTTGTACTATTCTCACCAGACCTACTAGGAGACCCAGACAACTACACCCCAGCA 720
HEW1      661:CTAATCCTTGTACTATTCTCACCAGACCTACTAGGAGACCCAGACAACTATACCCCAGCA 720
HEW2      661:CTAATCCTTGTACTATTCTCACCAGACCTACTAGGAGACCCAGACAACTATACCCCAGCA 720
HDU       661:CTAATCCTTGTACTATTCTCACCAGACCTACTAGGAGACCCAGACAACTACACCCCAGCA 720
              ********************************************** *********

HWZS      721:AACCCACTAAACACCCCACCCCATATTAAACCAGAATGATATTTCTTATTCGCCTACGCT 780
HLW       721:AACCCACTAAACACCCCACCCCATATTAAACCAGAATGATATTTCTTATTCGCCTACGCT 780
HJP2      721:AACCCACTAAACACCCCACCCCATATTAAACCAGAATGATATTTCTTATTCGCCTACGCT 780
HVN       721:AACCCACTAAACACCCCACCCCATATTAAACCAGAATGATATTTCTTATTCGCCTACGCT 780
HKR1      721:AACCCACTAAACACCCCACCCCATATTAAACCAGAATGATATTTCTTATTCGCCTACGCT 780
HRC       721:AACCCACTAAACACCCCACCCCATATTAAACCAGAATGATATTTCTTATTCGCCTACGCT 780
HX        721:AACCCACTAAACACCCCACCCCATATTAAACCAGAATGATATTTCTTATTCGCCTACGCT 780
HJH       721:AACCCACTAAACACCCCACCCCATATTAAACCAGAATGATATTTCTTATTCGCCTACGCT 780
HMS       721:AACCCACTAAACACCCCACCCCATATTAAACCAGAATGATATTTCTTATTCGCCTACGCT 780
HKR2      721:AACCCACTAAACACCCCACCCCATATTAAACGAGAATGATATTTCTTATTCGCCTACGCT 780
HJP1      721:AACCCACTAAACACCCCACCCCATATTAAACCAGAATGATATTTCTTATTCGCCTACGCT 780
HYN       721:AACCCACTAAACACCCCACCCCATATTAAACCAGAATGATATTTCTTATTCGCCTACGCT 780
HEW1      721:AACCCACTAAACACCCCACCCCATATTAAACCAGAATGATATTTCTTATTCGCCTACGCT 780
HEW2      721:AACCCACTAAACACCCCACCCCATATTAAACCAGAATGATATTTCTTATTCGCCTACGCT 780
HDU       721:AACCCACTAAACACCCCACCCCATATTAAACCAGAATGATATTTCTTATTCGCCTACGCT 780
              ****************************** ***************************

HWZS      781:ATCCTACGTTCAATTCCTAATAAACTAGGTGGAGTGCTAGCTCTAATAGCCTCCATCCTA 840
HLW       781:ATCCTACGTTCAATTCCTAATAAACTAGGTGGAGTGCTAGCTCTAGTAGCCTCCATCCTA 840
HJP2      781:ATCCTACGTTCAATTCCTAATAAACTAGGTGGAGTGCTAGCTCTAGTAGCCTCCATCCTA 840
HVN       781:ATCCTACGTTCAATTCCTAATAAACTAGGTGGAGTGCTAGCTCTAGTAGCCTCCATCCTA 840
HKR1      781:ATCCTACGCTCAATTCCTAATAAACTAGGTGGAGTGCTAGCTCTAGTAGCCTCCATCCTA 840
HRC       781:ATCCTACGTTCAATTCCTAATAAACTAGGTGGAGTGCTAGCTCTAATAGCCTCCATCCTA 840
HX        781:ATCCTACGTTCAATTCCTAATAAACTAGGTGGAGTGCTAGCTCTAATAGCCTCCATCCTA 840
HJH       781:ATCCTACGTTCAATTCCTAATAAACTAGGTGGAGTGCTAGCTCTAATAGCCTCCATCCTA 840
HMS       781:ATCCTACGTTCAATTCCTAATAAACTAGGTGGAGTGCTAGCTCTAATAGCCTCCATCCTA 840
HKR2      781:ATCCTACGTTCAATTCCTAATAAACTAGGTGGAGTGCTAGCTCTAATAGCCTCCATCCTA 840
HJP1      781:ATCCTACGTTCAATTCCTAATAAACTAGGTGGAGTGTTAGCTCTAGTAGCCTCCATCCTA 840
HYN       781:ATTCTACGTTCAATTCCTAATAAACTAGGTGGAGTGTTAGCTCTAGTAGCCTCCATCCTA 840
HEW1      781:ATTCTACGTTCAATTCCTAATAAACTAGGTGGAGTGTTGGCTCTAGTAGCCTCCATCCTA 840
HEW2      781:ATTCTACGTTCAATTCCTAATAAACTAGGTGGAGTGTTGGCTCTAGTAGCCTCCATCCTA 840
HDU       781:ATTCTACGTTCAATTCCTAATAAACTAGGTGGAGTGTTGGCCCTAGTAGCCTCCATCCTA 840
              ** ***** ************************* * ** *** **************
```

```
HWZS      841:ATCCTAATTTTAATGCCCATACTACACACATCCAAACAACGAAGCATAATATTTCGACCA 900
HLW       841:ATCCTAATTTTAATGCCCATACTACACACATCCAAACAACGAAGCATAATATTTCGACCA 900
HJP2      841:ATCCTAATTTTAATGCCCATACTACACACATCCAAACAACGAAGCATAATATTTCGACCA 900
HVN       841:ATCCTAATTTTAATGCCCATACTACACACATCCAAACAACGAAGCATAATATTTCGACCA 900
HKR1      841:ATCCTAATTTTAATGCCCATACTACATACATCCAAACAACGAAGCATAATATTTCGACCA 900
HRC       841:ATCCTAATTTTAATGCCCATACTACACACATCCAAACAACGAAGCATAATATTTCGACCA 900
HX        841:ATCCTAATTTTAATGCCCATACTACACACATCCAAACAACGAAGCATAATATTTCGACCA 900
HJH       841:ATCCTAATTTTAATGCCCATACTACACACATCCAAACAACGAAGCATAATATTTCGACCA 900
HMS       841:ATCCTAATTTTAATGCCCATACTACACACATCCAAACAACGAAGCATAATATTTCGACCA 900
HKR2      841:ATCCTAATTTTAATGCCCATACTACACACATCCAAACAACGAAGCATAATATTTCGACGA 900
HJP1      841:ATCCTAATTTTAATGCCCATACTACACACATCCAAACAACGAAGCATAATATTTCGACCA 900
HYN       841:ATCCTAATTTTAATGCCCATACTACACACATCCAAACAACGAAGCATAATATTTCGACCA 900
HEW1      841:ATCCTAATTTTAATGCCCATACTACACACATCCAAACAACGAAGCATAATATTCCGACCA 900
HEW2      841:ATCCTAATTTTAATGCCCATACTACACACATCCAAACAACGAAGCATAATATTCCGACCA 900
HDU       841:ATCCTAATTTTAATGCCCATACTACACACATCCAAACAACGAGGCATAATATTTCGACCA 900
                ************************ ************** ********** **** *

HWZS      901:CTAAGTCAATGCCTATTCTGAATACTAGTAGCAGACCTCATTACACTAACATGAATTGGA 960
HLW       901:CTAAGTCAATGCCTATTCTGAATACTAGTAGCAGACCTCATTACACTAACATGAATTGGA 960
HJP2      901:CTAAGTCAATGCCTATTCTGAATACTAGTAGCAGACCTCATTACACTAACATGAATTGGA 960
HVN       901:CTAAGTCAATGCCTATTCTGAATACTAGTAGCAGACCTCATTACACTAACATGAATTGGA 960
HKR1      901:CTAAGTCAATGCCTATTCTGAATACTAGTAGCAGACCTCATTACACTAACATGAATTGGA 960
HRC       901:CTAAGTCAATGCCTATTCTGAATACTAGTAGCAGACCTCATTACACTAACATGAATTGGA 960
HX        901:CTAAGTCAATGCCTATTCTGAATACTAGTAGCAGACCTCATTACACTAACATGAATTGGA 960
HJH       901:CTAAGTCAATGCCTATTCTGAATACTAGTAGCAGACCTCATTACACTAACATGAATTGGA 960
HMS       901:CTAAGTCAATGCCTATTCTGAATACTAGTAGCAGACCTCATTACACTAACATGAATTGGA 960
HKR2      901:CTAAGTCAATGCCTATTCTGAATACTAGTAGCAGACCTCATTACACTAACATGAATTGGA 960
HJP1      901:CTAAGTCAATGCCTATTCTGAATACTAGTAGCAGACCTCATTACACTAACATGAATTGGA 960
HYN       901:CTAAGTCAATGCCTATTCTGAATACTAGTAGCAGACCTCATTACACTAACATGAATTGGA 960
HEW1      901:CTAAGTCAATGCCTATTCTGAATACTAGTAGCAGACCTCATTACACTAACATGAATTGGA 960
HEW2      901:CTAAGTCAATGCCTATTCTGAATACTAGTAGCAGACCTCATTACACTAACATGAATTGGA 960
HDU       901:CTAAGTCAATGCCTATTCTGAATACTAGTAGCAGACCTCATTACACTAACATGAATTGGA 960
                ************************************************************

HWZS      961:GGACAACCCGTAGAACACCCATTCATCATCATCGGCCAACTAGCCTCCATCTTATATTTC 1020
HLW       961:GGACAACCCGTAGAACACCCATTCATCATCATTGGCCAACTAGCCTCCATCTTATATTTC 1020
HJP2      961:GGACAACCCGTAGAACACCCATTCATCATCATCGGCCAACTAGCCTCCATCTTATATTTC 1020
HVN       961:GGACAACCCGTAGAACACCCATTCATCATCATCGGCCAACTAGCCTCCATCTTATATTTC 1020
HKR1      961:GGACAACCCGTAGAACACCCATTCATCATCATCGGCCAACTAGCCTCCATCTTATATTTC 1020
HRC       961:GGACAACCCGTAGAACACCCATTCATCATCATCGGCCAACTAGCCTCCATCTTATATTTC 1020
HX        961:GGACAACCCGTAGAACACCCATTCATCATCATCGGCCAACTAGCCTCCATCTTATATTTC 1020
HJH       961:GGACAACCCGTAGAACACCCATTCATCATCATCGGCCAACTAGCCTCCATCTTATATTTC 1020
HMS       961:GGACAACCCGTAGAACACCCATTCATCATCATCGGCCAACTAGCCTCCATCTTATATTTC 1020
HKR2      961:GGACAACCCGTAGAACACCCATTCATCATCATCGGCCAACTAGCCTCCATCTTATATTTC 1020
HJP1      961:GGACAACCCGTAGAACACCCATTCATCATCATCGGCCAACTAGCCTCCATCTTATATTTC 1020
HYN       961:GGACAACCCGTAGAACACCCATTCATCATCATCGGCCAACTAGCCTCCATCTTATATTTC 1020
HEW1      961:GGACAACCCGTAGAACACCCGTTCATCATCATCGGCCAACTAGCCTCCATCTTATACTTC 1020
HEW2      961:GGACAACCCGTAGAACACCCGTTCATCATCATCGGCCAACTAGCCTCCATCTTATACTTC 1020
HDU       961:GGACAACCCGTAGAACACCCGTTCATCATCATCGGCCAACTAGCCTCCATCTTATACTTC 1020
                ******************* *********** ********************** ***
```

```
HWZS      1021:CTAATCATTCTAGTATTGATACCAAT 1046
HLW       1021:CTAATCATTCTAGTATTGATACCAAT 1046
HJP2      1021:CTAATCATTCTAGTATTGATACCAAT 1046
HVN       1021:CTAATCATTCTAGTATTGATACCAAT 1046
HKR1      1021:CTAATCATTCTAGTATTGATACCAAT 1046
HRC       1021:CTAATCATTCTAGTATTGATACCAAT 1046
HX        1021:CTAATCATTCTAGTATTGATACCAAT 1046
HJH       1021:CTAATCATTCTAGTATTGATACCAAT 1046
HMS       1021:CTAATCATTCTAGTATTGATACCAAT 1046
HKR2      1021:CTAATCATTCTAGTATTGATACCAAT 1046
HJP1      1021:CTAATCATTCTAGTATTGATACCAAT 1046
HYN       1021:CTAATCATTCTAGTATTGATACCAAT 1046
HEW1      1021:CTAATCATTCTAGTATTGATACCAAT 1046
HEW2      1021:CTAATCATTCTAGTATTGATACCAAT 1046
HDU       1021:CTAATCATTCTAGTATTGATACCAAT 1046
               *************************
```

Figure 2 Sequences of 1046bp of *Cyt* B gene fragment in fifteen haplotypes from exotic pig breeds

BIOGRAPHY

Mr. ShengLin Yang was born on October 5, 1963 in Guizhou province, China. He received Bachelor degree from Animal Science Department at Guizhou Agricultural College (it has been combined to Guizhou University) in 1985. In the same year, he was appointed a teaching assistant in animal Science Department. He was promoted to be a lecturer in 1996. In the past 22 years, he engaged in some teaching and research work regarding Animal Nutrition, Animal Production as well as Animal breeding. In December 2001, he was promoted to be an associate professor. From 2002 to 2003, he was invited to Zhejiang University in China and Massey University in New Zealand to do cooperative research work as a visiting scholar. In 2004, he obtained an opportunity to pursue a PhD program in Animal Breeding under the supervision of Dr. Pongchan Na-Lampang in school of Animal Production Technology, Suranaree University of Technology, Thailand. Until now, his eighteen research papers have been published.