การศึกษาการกระจายตัวของ Microsatellite DNA ในกลุ่มประชากรไทย

นาย ปิยะเชษฐ์ สตันยสุวรรณ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาวิทยาศาสตร์การแพทย์
คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2548

ISBN 974-53-2021-8

THE STUDY OF GEOGRAPHIC DISTRIBUTIONS OF MICROSATELLITE DNA IN THAI POPULATION

Mr. Piyachet Stanyasuwan

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Medical Science
Faculty of Medicine
Chulalongkorn University
Academic Year 2005

ISBN 974-53-2021-8

Thesis Title	The Study of Geographic Distribution of Microsatellite DNA in Thai Population
By	Mr. Piyachet Stanyasuwan
Field of Study	Medical Science
Thesis Advisor	Associate Professor Issarang Nuchprayoon, M.D.Ph.D
Thesis Co-advisor	Police lieutenant Colonal Kridsada Ribroumsub

---

Accepted by the Faculty of Medicine, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

P. Kamolratanakul ..... Dean of the Faculty of Medicine
(Professor Pirom Kamolratanakul, M.D.)

THESIS COMMITTEE

Apiwat Mutirangura ..... Chairman
(Professor Apiwat Mutirangura, M.D., Ph.D.)

Issarang Nuchprayoon ..... Thesis Advisor
(Associate Professor Issarang Nuchprayoon, M.D. Ph.D)

Pol Lt.Col K Ribroumsub ..... Thesis Co-advisor
(Police lieutenant Colonal Kridsada Ribroumsub)

Vorasuk Shotelersuk MD ..... Member
(Associate Professor Vorasuk Shotelersuk, M.D.)

ปิยะเชษฐ์ สตันยสุวรรณ การศึกษาการกระจายตัวของ Microsatellite DNA ในกลุ่มประชากรไทย. (THE STUDY OF GEOGRAPHIC DISTRIBUTIONS OF MICROSATELLITE DNA IN THAI POPULATION) อ.ที่ปรึกษา รศ.ดร.นพ อิศรางค์ นุชประยูร, อ.ที่ปรึกษาร่วม : พ.ต.ท.กฤษฎา ริบรวมทรัพย์ 66 หน้า. ISBN 974-53-2021-8.

ในปัจจุบันการพิสูจน์เอกลักษณ์บุคคล สามารถกระทำได้หลายวิธี โดยการตรวจลายพิมพ์ DNA เป็นอีกวิธีการหนึ่งโดยใช้การศึกษาในส่วนของ ช็อทแทนเดมรีพีท (short tandem repeat, STR) ซึ่งเป็นส่วนของ DNA ที่มีการเรียงซ้ำต่อๆ กัน โดยจำนวนหน่วยที่ซ้ำมี1-6 เบส STR เป็นบริเวณที่มีความหลากหลายสูงได้รับการถ่ายทอดจาก พ่อ แม่ ไปสู่ลูก และเป็นเอกลักษณ์เฉพาะบุคคล เพื่อให้เกิดค่าทางสถิติที่สามารถนำไปใช้คิดคำนวณทางนิติเวชศาสตร์ และเลือกใช้ตำแหน่ง STR ที่เหมาะสมกับประชากรไทย

การศึกษาโดยสกัดดีเอ็นเอจากเซลล์บนสำลีฟันปลายไม้ ซึ่งเช็ดเยื่อบุกระพุ้งแก้มของกลุ่มบุคคลตัวอย่างซึ่งไม่มีความสัมพันธ์เป็นเครือญาติ โดยแบ่งประชากรออกเป็นภูมิภาค คือ ภาคเหนือ อีสาน กลาง ตะวันออก ได้ ภูมิภาคละ 200 คน รวม 1,000 คนทั่วประเทศ แล้วทำการเพิ่มปริมาณสารพันธุกรรม โดยเทคนิค polymerase chain reaction (PCR) ที่ตำแหน่ง D8S1179, D21S11, D7S820, CSF1P0, D3S1358, THO1, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, Amelogenin, D5S818และ FGA จากนั้นนำ PCR product ที่ได้มาทำการแยกวิเคราะห์ด้วยเครื่อง Automate 3100 DNA Sequence

จากการศึกษาพบว่าในประชากรจำนวน 1,000 ราย ไม่พบว่ามีลายพิมพ์ดีเอ็นเอซ้ำกัน และ STR มีค่า Power of Discrimination ในช่วง 0.794 - 0.971, ค่า Matching Probability ในช่วง 0.029 - 0.206, ค่า PIC ในช่วง 0.755 - 0.86,ค่า Power of Exclusion ในช่วง 0.286 – 0.753, ค่า Typical Paternity Index ในช่วง 1.24 – 4.13, ค่า Heterozygosity ในช่วง 59.6 %- 87.9 % ดังนั้นตำแหน่ง STR ทั้งหมดที่ศึกษามีความหลากหลาย สามารถนำมาใช้ในการตรวจพิสูจน์เอกลักษณ์บุคคลโดยโอกาสที่บุคคลซึ่งไม่มีความสัมพันธ์ทางสายเลือดจะมีรูปแบบดีเอ็นเอเหมือนกันทั้ง 15 ตำแหน่งคือ 1 ใน 2.87×10^{17} คน และพบรูปแบบการกระจายของ STR ในภูมิภาคไม่แตกต่างกัน

สาขาวิชา.....วิทยาศาสตร์การแพทย์..... ลายมือชื่อนิสิต.....

ปีการศึกษา.....2548..... ลายมือชื่ออาจารย์ที่ปรึกษา.....

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

467 47464 30 : MAJOR MEDICAL SCIENCE
KEY WORD: MICROSATELLITE / SHORT TENDAM REPEAT (STRs)/ ALLELE

PIYACHET STANYASUWAN: THE STUDY OF GEOGRAPHIC DISTRIBUTIONS OF MICROSATELLITE DNA IN THAI POPULATION. THESIS ADVISOR: ASSOC. PROF. ISSARANG NUCHPRAYOON, M.D., PH.D, THESIS CO-ADVISOR: POL.LT. COL. KRIDSADA RIBROUMSUB, 66 pp. ISBN 974-53-2021-8

Short tandem repeat (STR) is valuable tool in Forensic Medicine for identification of individuals. Detailed statistics of each STR allele information is unique among each ethnic group. Frequency of each STR loci is needed for calculation of probability of match and has not been determined in a large scale in Thai population. It is not known whether STR loci may be able to identify people from each geographical region.

DNA from buccal swab from 1000 non-related Thais; 200 from each region: Northern, North-eastern, Eastern, Southern and Central of Thailand, were collected for PCR amplification of 16 STR loci, D8S1179, D21S11, D7S820, CSF1P0, D3S1358, THO1, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, Amelogenin, D5S818 and FGA, using AmpFlSTR® Identifiler™ kit. Automated 3100 DNA sequencer was used to analyze STR alleles using GeneMapper ID V3.2.

The results show difference of DNA fingerprint among the 1000 subject. The range of power of discrimination of STR is 0.794 - 0.971. The range of matching probability is 0.029-0.206. The range of polymorphic information content (PIC) is 0.55-0.86. The range power of exclusion is 0.286-0.753. The typical paternal index is 1.24-4.13. The heterozygosity is 59.6-87.6%. Hence all studied STR loci show variety and can be applied for identification of person and parent-and-child relationship. The chance of identify identical person using AmpFlSTR® Identifiler™ kit is 1 in 2.87×10^{17}. There is no distinctive pattern of any STR marker for people from each region of Thailand.

Field of study...Medical Science.....Student's signature.....

Academic year .....2005.....Advisor's signature.....

Co-advisor's signature.....

ACKNOWLEDGEMENTS

This thesis has been successful due to the valuable help, advice, suggestions and interest of my advisor, Associate Professor Dr. Issarang Nucnprayoon, Department of Pediatric, and my co-advisor, Police lieutenant Colonal Kridsada Ribroumsup Department of Forensic Service, Institute of Forensic Science Thailand, Ministry of Justice, to whom I would like to express my deep gratitude.

I would like to give my special thank to Miss Kamonwan kraitongsook, Miss Nongnuch Boonderm, Miss Chalampoo Wongvoravivat, Miss Sajee Imtang, Master Kortep Tepsitta for their help and cheerful. In addition, I would like to express my special thank to the competent officials of the Department of Forensic Service for providing me the conveniences.

Forthemore, this study was supported by the Molecular Biology Department of Research Affairs, Faculty of Medicine, Chulalongkorn University and Graduate School, Chulalongkorn University. Specials thanks to Ying, Ann, Tun, Yang, Pop, Not, Jeep and my friends who work on the 10^{th} floor of Chula MRC for their help, sincerity and friendship.

Finally, I will not forget to give special thanks to my parents and every members in my family for support during my graduate study and their kindness, understanding all the time; thank you very much.

CONTENTS

Page

LIST OF TABLES

Table Page

Table Page

LIST OF FIGURES

Figure Page

ABBREVIATION

DNA	Deoxyribonucleic acid
PCR	Polymerase chain reaction
VNTR	Variation number of tandem repeat
STR	Short tandem repeat
PI	Paternity Index
PE	Power of exclusion
Pm	Probability of matching
DP	Discrimination power
PIC	Polymorphism Information Content
PH	The negative logarithm of the concentration of hydrogen ions
rpm	Revolution per minute
Bp	Basepair
g	Gram
mg	Miiligram
µg	Microgram
ng	Nanogram
ml	Millilitre
µl	Microlitre
OD	Optical density
nm	Nanometre
cm	Centimetre
^{o}c	Degree Celsius
dNTP	Deoxy ribonucleotide triphosphate
Fig.	Figure

CHAPTER I

INTRODUCTION

Now a day, there are many anonymous dead bodies found. These bodies may be a part of any crime. The role of Forensic medicine is not only finding cause of death, but also to find out that who is the dead body. The objective for identifying person is not only for medical purpose. It can be use for much other purpose, as following.

1) Collect all information for people who are seeking for their dead cousin.
2) Find cause of death and record for statistic information.
3) Make the best management for the anonymous bodies by the information.
4) Make information of loosing person for legacy management.
5) Be information for insurance payment.
6) Help police working.

Sometime, identification of person does not need only medical knowledge. Examination of cloth, personal property, ornaments, document and medical evidence such as fingerprints, glasses, medicines are good evidence for the identification. Other medical devices, metal bone fixator, dental prosthesis, prosthetic heart valves, are also good evidences too. For conclusion of identification of person by anatomical and medical information can be explained as following.

1) Identify person by general information, such as age, sex, height, nationality. And combine with other evidence, such as cloth, ornament can make some clues. For example, we can find out sex of the bodies.
2) Identify person by comparison of ante-mortem and post-mortem information, such as dental history, skull X-rays, surgical history. From the disaster, Tsunami, at six provinces at southern Thailand. There are many lost and dead people. The victim numbers are about 5,300 bath Thai and foreigner. The victims have to be return to their families correctly.

So, person identification process must be done correctly too. There are three methods for person identification, as following.

1) Fingerprint is a very reliable method because fingerprint data had been collected by government. In this situation, this method is not so useful because the bodies had been damage by the disaster.

2) Forensic Odontology. is the third reliable and cheaper method. This method require dentist as specialist. The most important evidence is dental operation history. Unlike foreigner, many Thai people didn't have their dental history. Hence, this method is not suitable for Thai people.

3) DNA fingerprint, the new technique for identification, is another method. Because reliable statistic information of DNA fingerprint had not been collected enough to find probability or chance of non-relative person having same DNA fingerprint, which was called mating probability. This report has been done for the reliable statistic reference.[1-5]

Since, O.J. Simpson's wife was murder, DNA fingerprint became well known in justiciary process in USA. In Thailand, it was used for identification of parent and child relationship of a murdered medical student of Ramathibodi hospital, Ms. Jenjira Ploy-angunsri.

The human body contains many organ and tissue, such as blood, hair, liver, spleen, kidneys. These organs comprise over 3,000 billion cells. The basic structures of these cells are nucleus and cytoplasm, exception for red blood cells. Red blood cell don't contain nucleus component. The genetic materials, DNA, can be found in both nucleus and cytoplasm. They locate in chromosome and mitochondria, respectively. Human cells contain 46 chromosomes. They can be pair as 23 pairs of them. Autosome was named for 22 pairs of them. Another pair was called sex chromosome, which labeled as X and Y.

Sex chromosomes of female are XX, which they are XY in male. A half of human chromosomes are chromosome from maternal ovum and another half are from paternal sperm. Fertilization makes both half of chromosomes become a 46 chromosomes embryo. Because of absence of Y chromosome in ovum, the embryo inherits Y chromosome from

paternal sperm only. Then the embryo will develop to be a male fetus. This inherit type can only occur as father to son.

DNA comprises chemical composition. Four bases, Adenine (A), Thymine (T), Cytosine(C) and Guanine (G) become long strand and twist into chromosome.

Human genome consists of nuclear and mitochondrial part. The nuclear genome is double strands DNA, $3x10^9$ base-sized, which scatter in 23 pair of chromosome. But the nuclear DNA consists of non coding sequence, which contains 50,000-100,000 genes. They are 5-10 % of all genomes. Some parts of gene are non coding sequence and be eliminated, they are called Intron. Another parts are coding sequence, called Exon. And 80 % of genomes are non coding sequence and found in extragenic part. These areas have repetitive sequence which found 30 % in genome. The function of these repeated base is still unknown. These repeated bases have difference of size and repeating number. They can be grouped as interspersed repeats and tandem repeats. [6-10]

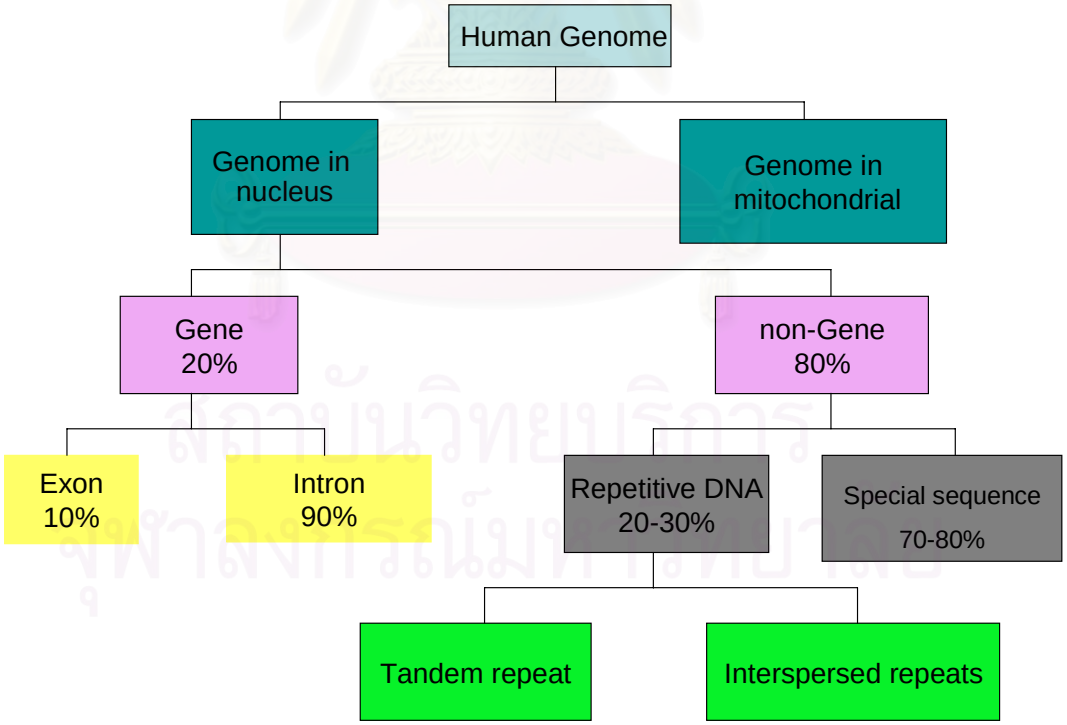

Fig. 1 Show Human Genome

Interspersed repeats are repeated base scattering in many area of genome. They are found separately, not the long sequence. They can be grouped by number of repeated base as short interspersed element, SINSE, and long interspersed element. They are separately 130-300 base sized in many genome and over 500 base sized, respectively. All are found 1-2% in genome.

Tandem repeats are bases which have long sequence. There are 3 type of them, satellite, minisatellite, and microsatellite.

Satellite is short repeated base 1-6 base sized. It also be long repeated hundreds base sized, which repeats 10^3-10^7 time for each locis. This type is highly repetitive DNA. Any type of satellite can be found 1 or 2 loci on chromosome, usually found at centromere. For example, satellite III is found at the first centromere and Y-chromosome which contain 5 base sized repeated base and consensus sequence that is TTCCA.

Minisatellite is 9-10 base-sized repeated bases. Repeated size can be over 10 time but not over than 1,000 time. This type is moderate repetitive DNA. Dr. Jeffery,et al, the first team who know how to identify DNA fingerprint by these repeated bases from their study. Many minisatellites have likeness of base sequence or some core sequence. Because of variety in some repeats base, they are called variable number of tandem repeats, VNTR.

Microsatellite is 1-6 base sized repeated base, such as $(A)_n$ $(CA)_n$ $(TAA)_n$ $(GaTA)_n$,when n is number of repeating. Numbers of repeating of each loci are not over than 100 time. Sometime, they are called simple sequence repeats (SSR) or short tandem repeats (STR). This type can be found on genome about 10^4-10^5 loci. Variability of these areas can be applied for identify DNA fingerprint. They are used for genome mapping because they are distributed on all over chromosome. [6-9, 11- 13]

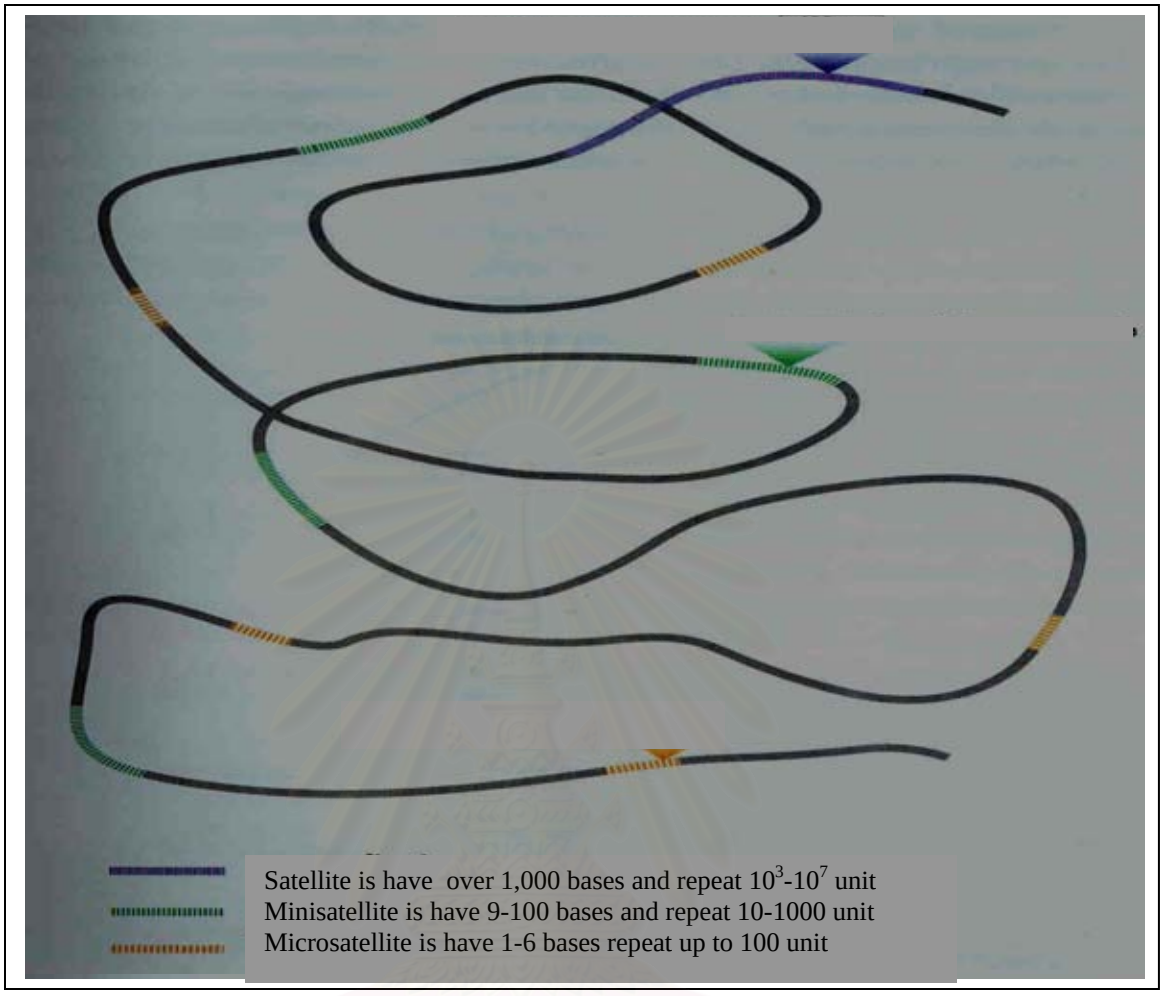

Fig.2 Type of Tandem repeats

Expected benefits are:

1. Understanding of the properties of allele in Thai population. How many types do they have? Which type is the most frequent allele? And does it have enough variety to be an identifiable tool in Forensic Medicine?
2. Understanding of allele's character of population in each region of Thailand. How many types of them? Which is the most frequent allele? So, the statistic probability of each region can be shown.
3. Explanation of evolution and migration of Thai population.
4. Applying for paternal and child relationship identification.
5. Applying to be biological evidences for Forensic Medicine.

CHAPTER II

LITERATURE REVIEW

Person identification and paternity testing based on blood group (ABO group) substances, red blood cell enzymes and HLA-DQα have a limitation. To circumvent this problem, many other human genetic markers were developed. One of these markers is STR. The polymorphic character of short tandem repeats (STR) loci results from variation in the number of tandemly repeated units from one allele to another. Different classes of STR loci have been described, including dinucleotides, trinucleotides and tetra nucleotides.[14-22]

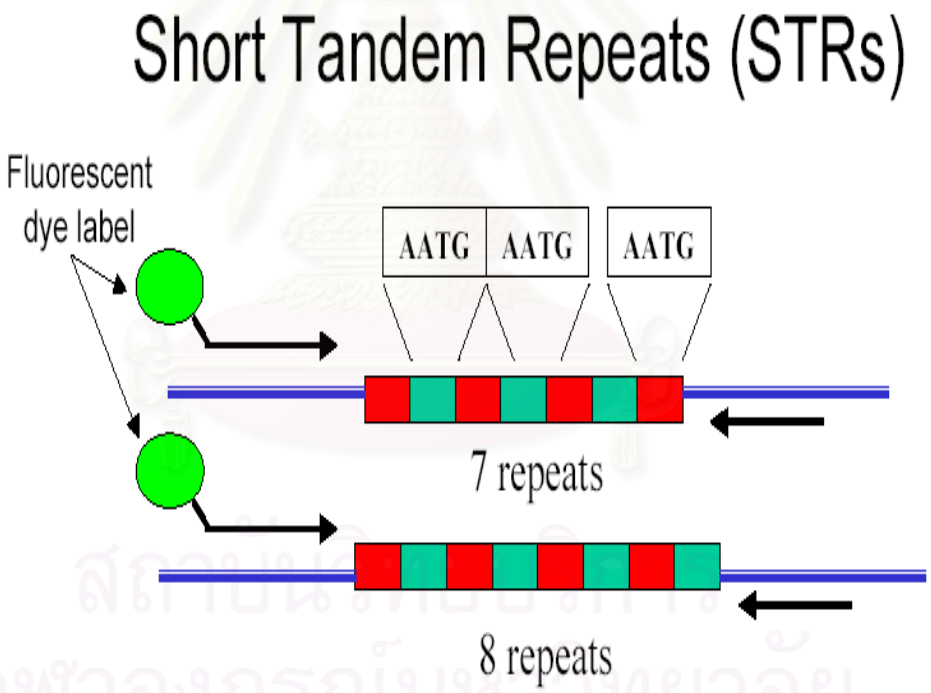

Fig. 3… Internal structure of short tandem repeats. AATG is tetranucleotides.

PCR-based STR markers provide several advantages for genetic mapping:

1. Ascertainment of genotypic data is easier and faster than with hybridization-based VNTR probes.

2. STRs often detect highly polymorphic loci.
3. STRs are abundant throughout the genome and are easy to develop as genetic markers.
4. The oligonucleotide-primer sequences flank the repeats. Tri and Tetranucleotide repeats show better stability during PCR than do dinucleotide repeats, the increased PCR stability facilitates interpretation of genotypes. The majority of the markers detected from human genetic maps contain tetranucleotide repeats. A lot of tetranucleotide repeats markers have been studied.

PCR technique for DNA fingerprint identification is preceded by 3 steps. 1.) Extraction of DNA. 2.) Duplication of DNA (PCR). 3.) Electrophoresis and recording the results.

16 loci for DNA fingerprint are used, they are D8S1179 D21S11 D7S820 CSF1P0 D3S1358 TH01 D13S317 D16S539 D2S1338 D19S433 vWA TPOX D18S51 Amelogenin D5S818 FGA. These loci are global reliable loci for identification of person. The test kits for these loci are called "AmpFlSTR® Identifiler™ PCR Amplification Kit (Applied Biosystems)" Each locus contain different repeated number. [23]

In 2001, Wasun Chantratita.;et al. studied 9 unlinked STR loci derived from Thai population group100 Unrelated individuals who requested for paternity testing at Ramathibade hospital. STR loci to studied used D3S1358 vWA FGA THO1 TPOX CSF1P0 D5S818 D13S317 and D7S820. The power of discrimination range from 0.8082 to 0.9653 results show that locus "FGA" has the highest power of discrimination; 0.9653.and locus "TPOX" has the lowest power of discrimination, 0.8082. [24]

In 2003, Miguel Angel Chiurillo et al they studied 15 loci of The AmpFlSTR® Identifiler™ PCR Amplification Kit (Applied Biosystems). The sample population contained 255 unrelated subjects from Caracas city, 206 with four grandparents born in Venezuela, and 49 randomly sampled regardless of the origin of grandparent. STR loci to studied used D8S1179 D21S11 D7S820 CSF1PO D3S1358 TH01 D13S317 D16S539 D2S1338 D19S433 vWA TPOX D18S51 D5S818 FGA. The power of discrimination range from 0.8733 to 0.9716 results show that locus "FGA" has the highest power of

discrimination; 0.9716.and locus “CSF1PO” has the lowest power of discrimination, 0.8733. The power of Exclusion range from 0.4313 to 0.8235 results show that locus “FGA” has the highest power of Exclusion; 0.8235.and locus “TPOX” has the lowest power of Exclusion, 0.4313. The probability of identity range from 0.0284 to 0.1253 results show that locus “TPOX” has the highest probability of identity; 0.1253.and locus “FGA” has the lowest probability of identity, 0.0284. [25]

Lay Hong Seah et al they studied 15 loci of The AmpFlSTR® Identifiler™ PCR Amplification Kit (Applied Biosystems). Compared for the three main ethnic groups of the Malaysian population comprising 210 Malays, 219 Chinese and 209 Indians. The combined probabilities of identity for the 15 STR loci are approximately 2.6×10^{-17},7.0×10^{-16} and 3.6×10^{-17}, respectively for the Malay, Chinese and Indian race groups. The combined probabilities of excluding paternity for the Malay, Chinese and Indian race groups are 0.9999980, 0.9999989 and 0.9999990, respectively. The power of discrimination range from 0.780 to 0.969 results show that locus “FGA” has the highest power of discrimination; 0.969.and locus “TPOX” has the lowest power of discrimination, 0.780 The power of Exclusion range from 0.314 to 0.738 results show that locus “D21S11” has the highest power of Exclusion; 0.738.and locus “TPOX” has the lowest power of Exclusion, 0.314. [26]

Anna Barbaro et al they studied 15 loci of The AmpFlSTR® Identifiler™ PCR Amplification Kit (Applied Biosystems).three populations from Southern Italy (Calabria): Reggio Calabria, Catanzaro, and Cosenza. [27]

Yoo-Li Kim, et al they studied 15 loci of The AmpFlSTR® Identifiler™ PCR Amplification Kit (Applied Biosystems). Populations 231 unrelated individuals living in Korea. The combined PD and PE for the 15 STR loci in a Korean population were 2.85×10^{-17} and0.9999968, respectively. When we compared the data with the value from other populations supplied by PE Applied Biosystems It showed that this AmpFlSTR multiplex system in Korean has lower forensic capacity than Caucasian and African. However, it has similar forensic capacity with Native American. The power of discrimination range from 0.818 to 0.965 results show that locus “D2S1338” has the highest power of discrimination; 0.965.and locus “TPOX” has the lowest power of discrimination, 0.818.

The power of Exclusion range from 0.298 to 0.744 results show that locus “D18S51” has the highest power of Exclusion; 0.744.and locus “TPOX” has the lowest power of Exclusion, 0.298. The Typical paternity index range from 1.27 to 3.98 results show that locus “D18S51” has the highest Typical paternity index; 3.98.and locus “TPOX” has the lowest Typical paternity index, 1.27. The Matching probability range from 0.035 to 0.182 results show that locus “TPOX” has the highest Matching probability; 0.182.and locus “D2S1338” has the lowest Matching probability, 0.035 [28]

In 2004, Witold Pepinski et al they studied 15 loci of The AmpFlSTR® Identifiler™ PCR Amplification Kit (Applied Biosystems) population136 sample of Old Believers living in the northeastern Poland for the use as a highly discriminatory system of genetic markers in population studies and in personal identification. The power of discrimination range from 0.807 to 0.967 results show that locus “D18S51” has the highest power of discrimination; 0.967.and locus “TPOX” has the lowest power of discrimination, 0.807. The power of Exclusion range from 0.243 to 0.714 results show that locus “FGA” has the highest power of Exclusion; 0.714.and locus “TPOX” has the lowest power of Exclusion, 0.243. The Typical paternity index range from 1.13 to 4.43 results show that locus “D2S1338” has the highest Typical paternity index; 4.43.and locus “TPOX” has the lowest Typical paternity index, 1.13. The Matching probability range from 0.193 to 0.033 results show that locus “TPOX” has the highest Matching probability; 0.193.and locus “D2S1338” has the lowest Matching probability, 0.033 [29]

J.A. Morales et al they studied 15 loci of The AmpFlSTR® Identifiler™ PCR Amplification Kit (Applied Biosystems). in an El Salvadoran sample population, consisting of 228 unrelated individuals All loci are highly polymorphic with the locus D3S1358 having the lowest observed heterozygosity (Ho) (64.9%) and loci D18S51 and FGA both displaying the highest heterozygosity (86.4%). The power of discrimination range from 0.852 to 0.967 results shows that locus “FGA” has the highest power of discrimination; 0.967.and locus “TPOX” has the lowest power of discrimination, 0.852. The power of Exclusion range from 0.354 to 0.723 results shows that locus “FGA and D18S51” has the highest power of Exclusion; 0.723.and locus “D3S1358” has the lowest power of Exclusion, 0.354[30]

M. Kubat et al they studied 15 loci of The AmpFlSTR® Identifiler™ PCR Amplification Kit (Applied Biosystems). In a sample of 136 unrelated Albanian adults from Kosovo. The power of discrimination range from 0.816 to 0.964 results show that locus “FGA” has the highest power of discrimination; 0.964.and locus “TPOX” has the lowest power of discrimination, 0.816. The power of Exclusion range from 0.415 to 0.760 results show that locus “D18S51” has the highest power of Exclusion; 0.760.and locus “TPOX” has the lowest power of Exclusion, 0.415. The Matching probability range from 0.036 to 0.184 results show that locus “TPOX” has the highest Matching probability; 0.184.and locus “FGA” has the lowest Matching probability, 0.036 [31]

M.V. Santos et al they studied 15 loci of The AmpFlSTR® Identifiler™ PCR Amplification Kit (Applied Biosystems). In 150 unrelated individuals from Bahia, Brazil

The power of discrimination range from 0.865 to 0.977 results show that locus “D2S1338” has the highest power of discrimination; 0.977.and locus “TPOX” has the lowest power of discrimination, 0.865. The power of Exclusion range from 0.466 to 0.774 results show that locus “D2S1338” has the highest power of Exclusion; 0.774.and locus “TPOX” has the lowest power of Exclusion, 0.466 [32]

.

Table.1 Show Literature Review Step

Year	Scientist	Population	Loci	Descriptive
2001	Rerkamnuaychoke	Thai 100 unrelated	D3S1358 vWA FGA THO1 TPOX CSF1P0 D5S818 D13S317 and D7S820.	Power of discrimination FGA= 0.9653 TPOX =0.8082
2003	Miguel Angel Chiurillo	Venezuela, 255 unrelated	AmpFlSTR® Identifiler™ PCR Amplification Kit (Applied Biosystems)	Power of discrimination FGA= 0.9716 CFS1PO =0.8733 Power of Exclusion FGA=0.8235 TPOX=0.4313
	Lay Hong Seah	Malaysian population comprising 210 Malays, 219 Chinese and 209 Indians.	AmpFlSTR® Identifiler™ PCR Amplification Kit (Applied Biosystems)	probabilities of excluding Malay, Chinese and Indian race groups are 0.9999980, 0.9999989 and 0.9999990
	Yoo-Li Kim	Korea Populations 231 unrelated	AmpFlSTR® Identifiler™ PCR Amplification Kit (Applied Biosystems)	Power of discrimination D2S1338 = 0.965 TPOX =0.818 Power of Exclusion D18S51=0.744 TPOX=0.298
2004	Witold Pepinski	population136 sample of Old Believers living in the northeastern Poland	AmpFlSTR® Identifiler™ PCR Amplification Kit (Applied Biosystems)	Power of discrimination D18S51 = 0.967 TPOX =0.807 Typical paternity index D2S1338 = 4.43 TPOX =1.13
	J.A. Morales	El Salvadoran sample population, consisting of 228 unrelated	AmpFlSTR® Identifiler™ PCR Amplification Kit (Applied Biosystems)	Heterozygosity D3S1358=64.9% D18S51,FGA=86.4%

CHAPTER III

MATERIALS AND METHODS

1. Specimens

Buccal Swab from volunteer to contact at Insititual of Forensic Science Classified data into 5 groups by the region which are North Center North-East and South. Then, and randomly by selected 200 samples form each region and used them as the Representative for each one by distributions is 5 area is North (Chiang Mai , Chiang Rai, Kamphaeng Phet, Lampang, Lamphun, Mae Hong Son, Nan, Phayao, Phetchabun, Phichit, Phitsanulok, Phrae, Sukhothai, Tak, Uttaradit) Center (Ang Thong, Ayutthaya, Bangkok, Chachoengsao, Chai Nat, Kanchanaburi, Lopburi, Nakhon Nayok, Nakhon Pathom, Nakhon Sawan, Nonthaburi, Pathum Thani, Phetchaburi, Prachuapkhirikhan, Prachin Buri, Ratchaburi, Samut Prakan, Samut Songkhram, Samut Sakhon, Saraburi, Singburi, Suphan Buri, Uthai Thani) North-East (Amnat Charoen, Buri Ram, Chaiyaphum, Kalasin, Khon Kaen, Loei, Maha Sarakham Mukdahan, Nakhon Phanom, Nakhon Ratchasima, Nong Khai, Nong Bua Lamphu, Roi Et, Sakon Nakhon, Si Sa Ket, Surin, Udon Thani, Ubon Ratchathani, Yasothon) East (Chanthaburi, Chon Buri, Rayong, Sa Kaeo, Trat) South (Chumphon, Krabi, Narathiwat, Nakhonsithammarat, Pattani, Phangnga, Phatthalung, Phuket, Ranong Satun, Songkhla, Surat Thani, Trang, Yala)

2. Materials

2.1 Microcentrifuge tubes, 1.5 ml. : (Axygen, USA)

2.2 PCR tubes, 0.2 ml.: (Axygen, USA)

2.3 Glass pipettes

2.4 Aerosol resistant pipette tips 2 µl, 10 µl, 20 µl, 100 µl, 200 µl, 1000 µl: Pipetman Renin

2.5 Beakers, Flasks, Volumetric Flasks,Cylinders, Reagent bottles

2.6 Disposable gloves

2.7 Microcentrifuge tube racks

2.8 Large metal binder clamps

2.9 Paper towel

2.10 Plastic Trays

2.11 Parafilm

2.12 Plastic wrap

3. Equipment

3.1 Heat block: (Multi-Block® Heater, LAB-NET)

3.2 Microcentrifuge : (Labofuge 300 Heraeus)

3.3 Autoclave: (Astell)

3.4 pH meter: (CONSORT C832)

3.5 Vortex: (VX 100 Labnet)

3.6 Microwave: (SHARP)

3.7 Refrigerator 4°C: (SAWDEN INTERCOOL)

3.8 Freezer -20°C: (Reveo)

3.9 Thermal cycle: (GeneAmp® PCR System 9700)

3.10 Laminar flow hood: (HEAL FORCE)

3.11 Fume hood: (ESCO SMART CONTROL)

3.12 Automate Sequence: (3100 Genetic Analyzer HITACHI)

3.13 Thermometer: (CONSORT C832)

3.14 Automatic adjustable micropipettes; P2(0.1-2 µl), P10 (0.5-10µl), P20 (5-20µl), P200 (20-200µl), P1000 (100-1000µl): (Renin)

3.15 Hot plate with magnetic field, stirring-magnetic bar :(NW HP240)

4. Reagents

4.1 General reagents

4.2 Reagent kits

- AmpFèSTR® Identifiler™ PCR Amplification Kit (Applied Biosystems)
  - AmpFlSTR® PCR Reaction Mix
  - AmpFlSTR® Identifiler™ Primer Set
  - AmpliTaq Gold® DNA Polymerase
  - AmpFlSTR® Control DNA 9947A
  - AmpFlSTR® Identifiler™ Allelic Ladder
- POP4™ Polymer (Applied Biosystems)

- Hi-Di^{TM} Formamide for 3100 Genetic Analyzer
- GeneScan-500 LIZ Size Standard

4.3 Enzymes

Protenase K

5. Methods

5.1 Sample collection

Cotton swab form buccal 10 second from left buccal and 10 second form right buccal

5.2 DNA extraction

1. Suspend the swab sample in 1 mL of autoclaved deionized water in an autoclaved 1.5-mL microcentrifuge tube.

2. Incubate at room temperature for 30 minutes to dehydrate the sample.

3. Use an autoclaved toothpick to tease the fibers apart on the inside of the tube. Twirl the swab and toothpick for two minutes to release the cells from the swab.

4. Remove the substrate and toothpick. Spin in a microcentrifuge for two minutes at 10,000–15,000 µ*g* (maximum speed) at room temperature

5. Without disturbing the pellet, remove and discard all but 25 µL (or twice the volume of the pellet, whichever is greater) of the supernatant.

6. Resuspend the pellet in the remaining 25 µL by stirring with an autoclaved pipette tip.

7. Add 5% Chelex to a final volume of 200 µL

8. Add 2 µL of 10 mg/mL Proteinase K. Mix gently.

9. Incubate the sample at 56 °C for 15–30 minutes.

10. Vortex the sample at high speed for 5–10 seconds.

11. Spin the sample in a microcentrifuge for 10–20 seconds at 10,000–15,000 x *g* (maximum speed) at room temperature.

12. Incubate the sample in a boiling water bath for eight minutes.

13. Vortex the sample at high speed for 5–10 seconds.

14. Spin the sample in a microcentrifuge for 2–3 minutes at 10,000–15000x *g* (maximum speed) at room temperature. The sample is ready for DNA quantitation and the PCR

15. Store the remainder of the sample at either 2–6 °C or –15 to –25 °C.

5.3 Calculation of DNA concentration

The OD reading at 260 nm is used for calculating the concentration of nucleic acid in the samples. An OD of 1correspond to approximately 50 μg/ml for double-stranded DNA. Therefore, DNA concentration can be calculated from the following formula

DNA concentration = OD X 50 X dilution ratio (μg/ml)

Dilute an aliquot of the original DNA to obtain the working DNA solution (working concentration approximately 0.10 ng/μl)

5.4 PCR Amplification

The amplification of multiplex reactions of D8S1179, D21S11, D7S820, CSF1P0, D3S1358, THO1, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, Amelogenin, D5S818, FGA was performed according to the manufacturer's recommendations using the AmpFlSTR® Identifiler™ kit

The use of gloves and aerosol-resistant pipette tips is essential to prevent cross-contamination.

The setup of the PCR and the following electrophoresis analysis of the amplification reactions were carried out in two separate rooms. During amplification set-up, all of the reaction tubes were kept on ice.

To determine the number of reactions to be set up, this should include one positive and one negative control reaction tube. Add 1 or 2 reaction volumes to this number to compensate for pipetting errors. While this approach does waste a small amount of each reagent, it ensures that you will have enough PCR Master Mix for all samples.

1. Determine the total number of samples, including controls and negative.
2. Vortex the following reagents for 5 sec:
    - ♦AmpFlSTR PCR Reaction Mix
    - ♦AmpliTaq Gold DNA Polymerase
    - ♦AmpFlSTR Identifiler Primer Set

3. Calculate the required amount of components as shown:

   Number of samples X 10.5 μL of AmpFlSTR PCR Reaction Mix

   Number of samples X 0.5 μL of AmpliTaq Gold DNA Polymerase

   Number of samples X 5.5 μL of AmpFlSTR Identifiler Primer Set

4. Vortex the master mix at medium speed for 5 sec.
5. Dispense 15 μL of master mix per PCR tube.
6. DNA test sample tube and the sample DNA concentration is≤ 0.125 ng/μL
7. Add 10 μL of sample to the PCR tube.
8. Place the PCR tube in the Thermal cycle. (GeneAmp® PCR System 9700)

Initial Incubation Step	Denature	Anneal	Extend	Final Extension	Final Step
HOLD	CYCLE (28 cycles)			HOLD	HOLD
95 °C 11 min	94 °C 1 min	59 °C 1 min	72 °C 1 min	60 °C 60 min	4–25 °C (forever)

- Table 2. Locus-Specific Information

STR locus	Chromosomal location	Repeats Sequence 5’ – 3’	STR ladder alleles	Control DNA 9947A
D8S1179	8	-	8-19	13,13
D21S11	21q11.2-q21	TCTA	24,24.2,25-28,28.2,29,29.2,30,30.2,31,31.2,33,33.2,34.34.2,35,35.2,36-38	30,30
D7S820	7q11.21-22	GATA	6-15	10,11
CSF1PO	5q33.3-34	AGAT	6-15	10,12
D3S1358	3q	TCTA	12-19	14,15
THO1	11p15.5	AATG	4-9,9.3,10,11,13.3	8,9.3
D13S317	13q22-31	GATA	8-15	11,11
D16S539	16q24-qter	GATA	5,8-15	11,12
D2S1338	2q35-37.1	AGAT	15-28	19,23
D19S433	19q12-13.1	-	9-12,12.2,13,13.2,14,14.2,15,15.2,16,16.2,17,17.2	14,15
vWA	12q12-pter		11-24	17,18
TPOX	2q23-2per	AATG	6-13	8,8
D18S51	18q21.3	GAAA	7,9,10,10.2,11-13,13.2,14,14.2,15-27	15,19
Amelogenin	X:p22.1-22.3 Y:p11.2	-	X,Y	X,X
D5S818	5q21-31	AGAT	7-16	11,11
FGA	4q28	CTTT	17-26,26.2,27-30,30.2,31.2,32.2,33.2,42.2,43.2,44.2,45.2,46.247.2,48.2,50.2,51.2	23,24

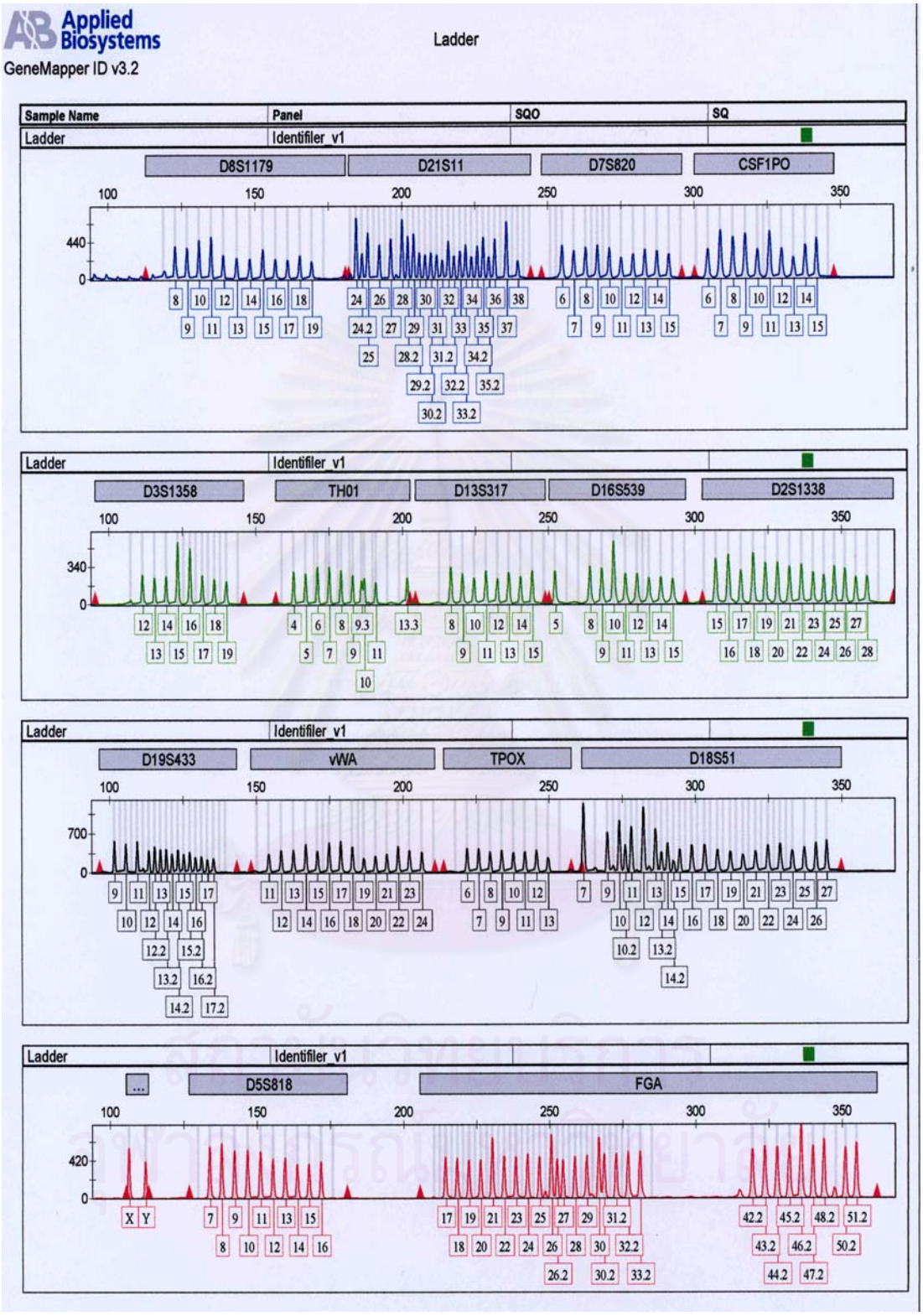

Fig.4 Show ladder of STR of AmpFlSTR® Identifiler™ PCR Amplification Kit (Applied Biosystems)

5.5 Gel electrophoresis and Genotype determination

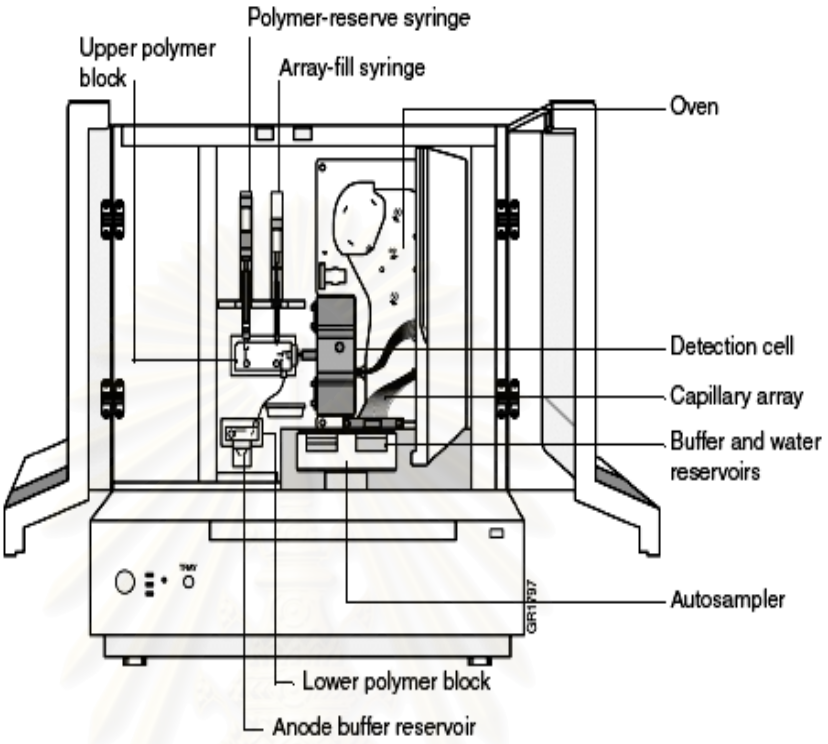

Fig. 5 Component of 3100 Genetic Analyzer

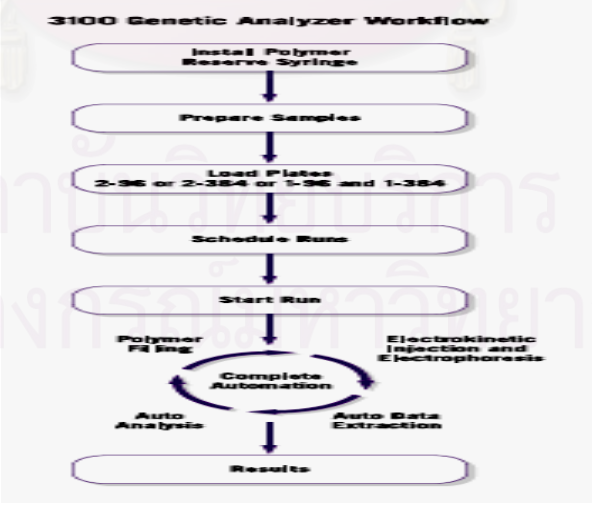

Fig. 6 Process step for 3100 Genetic Analyzer

- To Install Polymer to syringe:
    1. Prime the syringe with approximately 0.1 mL of POP-4 polymer.
    2. Fill the 1.0-mL syringe manually with a maximum of 0.8 mL of POP-4 polymer.
    3. Wipe the outside of syringe with a Kim wipe tissue to dry.
    4. Remove any air bubbles by inverting the syringe and pushing a small amount of polymer out of the tip.
- Prepare sample
    1. Combine the necessary amount of Hi-Di™ Formamide and GeneScan™-500 LIZ™ Size Standard in a single microcentrifuge tube as shown:
        - ♦(Number of samples + 2) × 24.5 µL Hi-Di Formamide
        - ♦(Number of samples + 2) × 0.5 µL GeneScan-500 LIZ Size Standard
    2. Vortex the tube to mix and Spin the tube briefly in a microcentrifuge.
    3. Aliquot 25 µL of Hi-Di Formamide/GeneScan-500 LIZ solution into 0.2-mL or 0.5-mL Genetic Analyzer sample tubes.
    4. Add 1.5 µL of PCR product or AmpFlSTR Identifiler Allelic Ladder per tube. Mix by pipetting up and down.
    5. Seal each tube with a septum.
    6. Denature each sample for 3 min at 95 °C.
- To run the samples

The 3100 Genetic Analyzer Data Collection Software v 2.1 must be installed for use with the AmpFlSTR Identifiler PCR Amplification Kit.

- To Collect Result

The GeneScan Analysis Software sample data v3.7 and Genotyper® software v3.7 or GeneMapper ID v3.2

5.6 Statistical analysis

1. The frequency of each allele for each STR was calculated by dividing the observed number of a specific allele by the total number of allele.

2. Heterozygotes are individuals with different alleles at the same locus. Homozygotes are individuals with two identical alleles at a given locus. Observed heterozygosity is also called frequency of heterozygotes and is represented by **h** in the following equation

$$h = nh / n$$

where nh is the number of individual observations with two alleles and n is the total number of individuals.

Since one is either a homozygote or a heterozygote, the frequency of heterozygote (h) plus the frequency of homozygote (H) is equal to one.

$$H + h = 1$$

The unbiased estimate of the expected heterozygosity (h) was calculated from the formula

$$h = n(1-\sum x^2) / (n-1)$$

where x^2 = allele frequency and n = total number of alleles observed.

3. The average power of exclusion is the probability that a falsely accused putative father will be excluded as the biological father of a particular child. The higher the probability, the better the genetic system for parentage testing. This probability can bee calculated for a trio when the genotypes of the alleged father, the mother and mother and child are known, and can be calculated also for a parent/child duo. The general formulae for both situations are given below

$$\text{PE trio} = \sum Pi(1-Pi)^2 + \sum (PiPj)^2(3Pi+3Pj-4)$$

$$\text{PE duo} = \sum Pi^2(1-Pi)^2 + \sum 2PiPj\,(1-Pi-Pj)^2$$

where Pi = most common allele , Pj = next most common allele .

The combine power of exclusion for multiple loci can be calculated by the following equation

$$\text{PE combined} = 1 - \prod(1-PE_i)$$

where n = the number of STR systems.

4. The probability of matching is the probability that two individuals selected at random form a given population will genotypically match at a particular locus or set of loci. The smaller the value of Pm, the batter the system for individualization purposes. This probability, Pm, is defined by the following formula

$$Pm = \sum P_i$$

where P_i represents the frequenceies of all possible genotypes

The combined matching probability for more than one locus is the product of the individual matching probability at each locus, assuming they are not linked

5. The discrimination power (DP) is the probability for to individuais chosen at random from a population to have different genotypes.

$$DP = \text{combined} = 1 - \sum(\text{expected genotype frequencies})^2$$

The combined power of discrimination for multiple loci can be calculated by the following equation :

$$\text{DP combined} = 1 - \prod (1-DP_i)$$

Where n = the number of STR system

6. The paternity index reflects how many more times likely it is that the person being tested is the biological father, rather than a randomly selected individual. The typical paternity index is assigned to a locus rather than an individual case. Generally , a $PI_{typical}$ is represented by the following equation :

$$PI_{typical} = 1/2H$$

where H is the frequency of homozygotes (in this study the frequency of expected homozygotes).

7. The polymorphism information content (PIC) measures how informative a marker is. The PIC of a marker is given by

$$PIC = 1- \sum Pi^2 - \sum \sum 2Pi^2 Pj^2$$

where Pi is the frequency of the i th allele

A marker with a PIC of 0 is never informative; one with a PIC of 1 is always informative.

CHAPTER IV

RESULTS

DNA extraction

DNA extracted by this method exhibits a 260/280 ratio above 1.6. The amount of DNA from Buccal Swab sample in the individual extract, calculatedfrom OD at 260 nm, ranges from 11.335 to 82.98 μg. Amplification of DNA from the sample was successful. The advantages of the salting-out methodare: rapid, inexpensive, avoiding the use of hazardous reagents and requiring few steps. It can be routinely used on Buccal Swab samples in laboratories for person identification and paternity test based on STR and PCR technology. However,several pipetting steps are required and thus several pipette tips are introduced into the samples. Besides, the steps followed lysis and washing the contamination thus decreasing the yields. Therefore it should be ensured that cross-contamination has not occurred.

PCR amplifications

Each locus described contains tetrameric short tandem repeats,the polymorphisms of which are due to the variable number of repeats of four nucleotide sequences All samples were successfully amplified for fifteen STR loci using the protocol supplied in the AmpFlSTR® Identifiler™ PCR Amplification Kit (Applied Biosystems) by combining one multiplex reaction with fifteen single-1ocus PCR. Still, all of these samples success to be amplified in the first attempt. There was no evidence of contamination throughout the whole study.

Electrophoresis

All samples were successfully amplified for fifteen STR loci using the protocol supplied in the AmpFlSTR® Identifiler™ PCR Amplification Kit (Applied Biosystems) all of these samples success to be Electrophoresis in the first attempt. There was no evidence of contamination throughout the whole study.

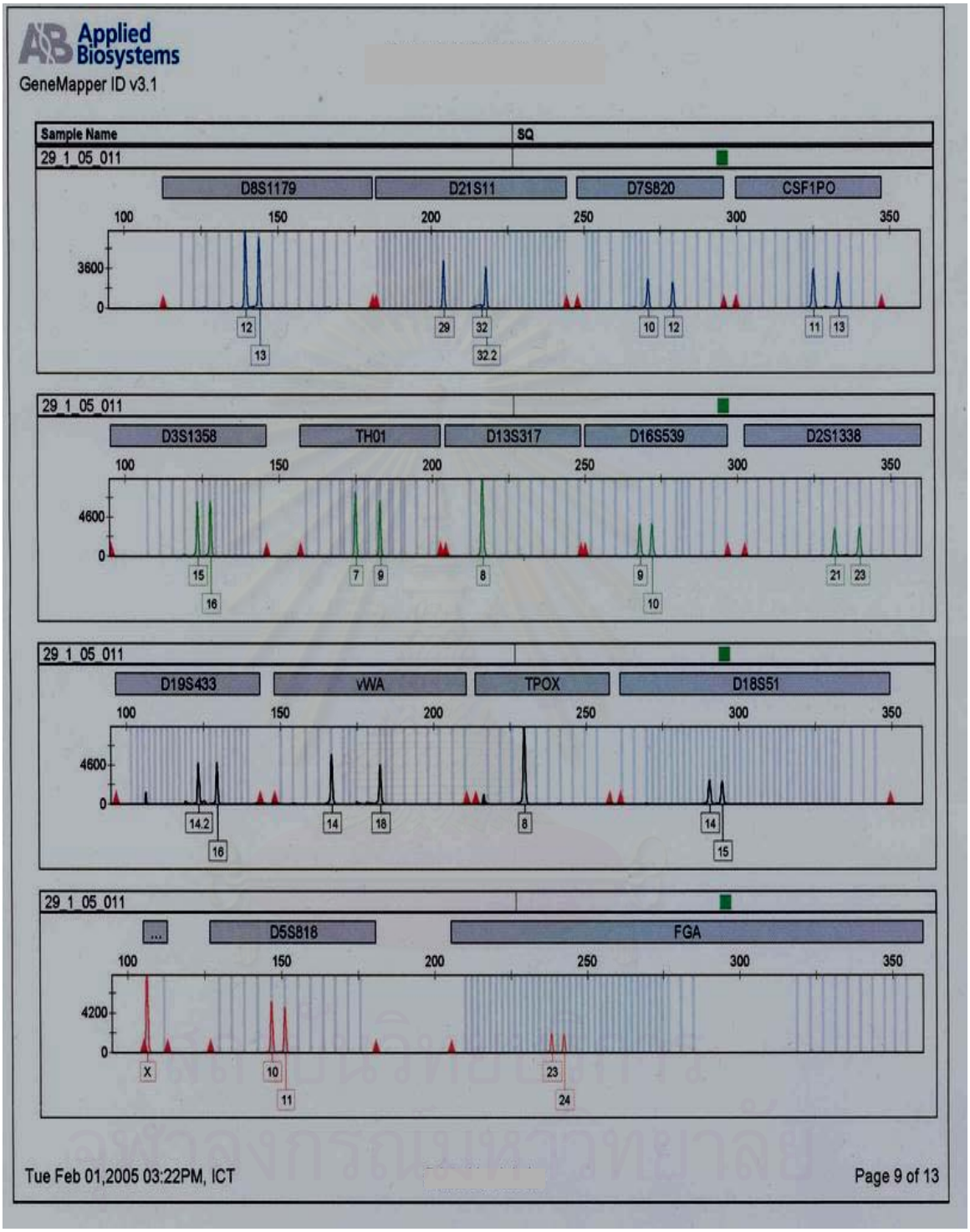

Fig. 7 Show Result 1 case from 3100 Genetic Analyzer by GeneMapper ID v3.1

Statistical analysis

All statistical analysis show in table

Table. 3 Statistics for Forensic identification and Parentage studies at Locus D8S1179

Locus D8S1179		ALL	North	Center	North East	East	South
Forensic	Matching Probability	0.042	0.051	0.043	0.048	0.045	0.048
	Power of Discrimination	0.958	0.949	0.957	0.953	0.955	0.952
	PIC	0.84	0.83	0.84	0.83	0.83	0.84
Paternity	Power of Exclusion	0.743	0.735	0.725	0.745	0.705	0.806
	Typical Paternity Index	3.97	3.85	3.70	4.00	3.45	5.26
Allele Frequencies	Homozygotes	12.6%	13.0%	13.5%	12.5%	14.5%	9.5%
	Heterozygotes	87.4%	87.0%	86.5%	87.5%	85.5%	90.5%
Total Alleles		2000	400	400	400	400	400

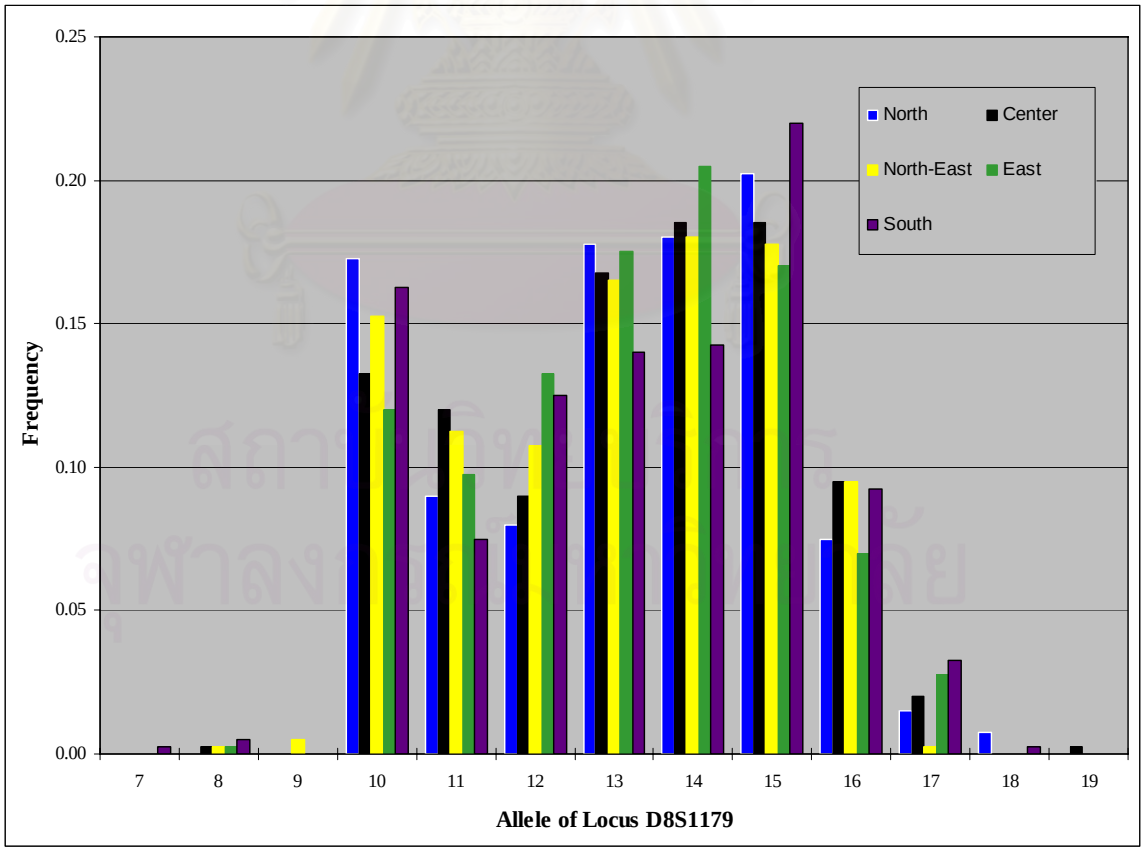

Fig. 8 The expected Allele Frequency of locus D8S1179

Table. 4 Statistics for Forensic identification and Parentage studies at Locus D21S11

Locus D21S11		ALL	North	Center	North East	East	South
Forensic	Matching Probability	0.047	0.069	0.045	0.056	0.051	0.045
	Power of Discrimination	0.953	0.931	0.955	0.944	0.949	0.955
	PIC	0.82	0.79	0.83	0.81	0.81	0.83
Paternity	Power of Exclusion	0.673	0.627	0.685	0.725	0.599	0.735
	Typical Paternity Index	3.11	2.70	3.23	3.70	2.50	3.85
Allele Frequencies	Homozygotes	16.1%	18.5%	15.5%	13.5%	20.0%	13.0%
	Heterozygotes	83.9%	81.5%	84.5%	86.5%	80.0%	87.0%
Total Alleles		2000	400	400	400	400	400

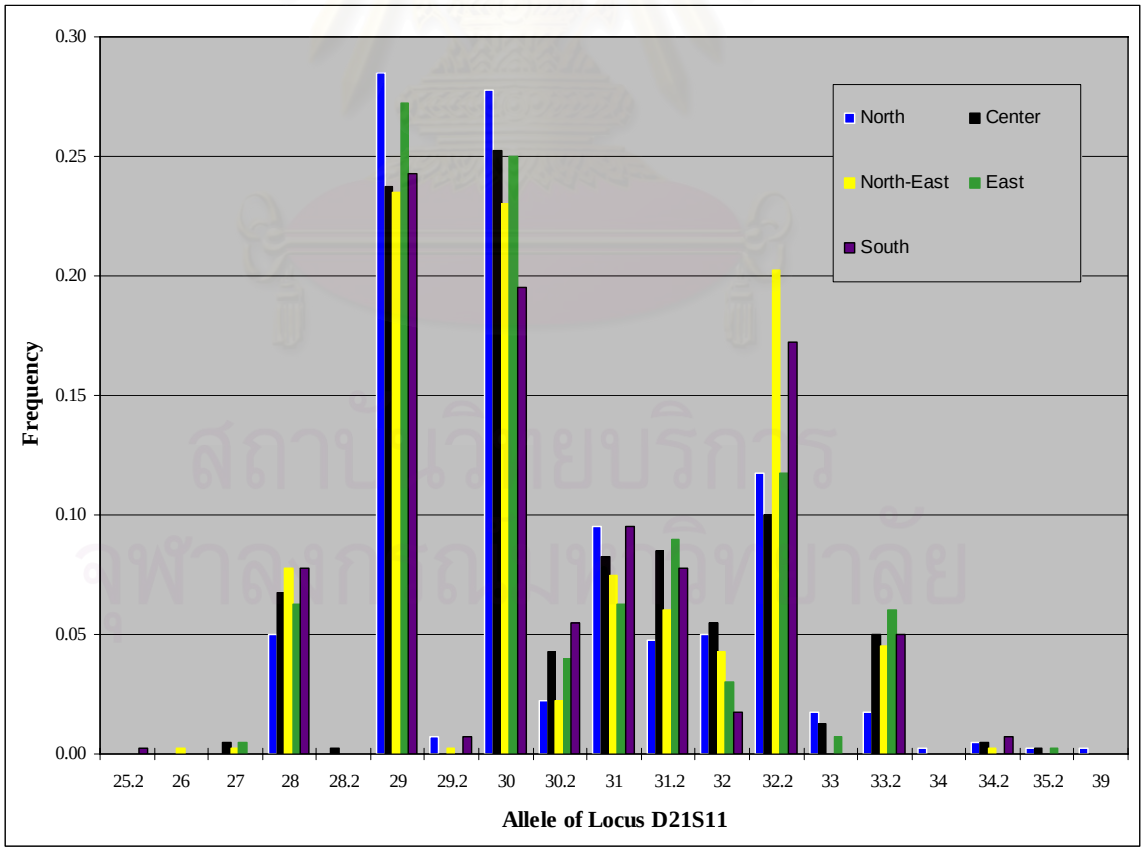

Fig. 9 The expected Allele Frequency of locus D21S11

Table. 5 Statistics for Forensic identification and Parentage studies at Locus D7S820

Locus D7S820		ALL	North	Center	North East	East	South
Forensic	Matching Probability	0.085	0.087	0.099	0.090	0.094	0.079
	Power of Discrimination	0.915	0.913	0.901	0.910	0.906	0.921
	PIC	0.74	0.75	0.72	0.75	0.72	0.76
Paternity	Power of Exclusion	0.525	0.581	0.444	0.599	0.428	0.590
	Typical Paternity Index	2.07	2.38	1.72	2.50	1.67	2.44
Allele Frequencies	Homozygotes	24.1%	21.0%	29.0%	20.0%	30.0%	20.5%
	Heterozygotes	75.9%	79.0%	71.0%	80.0%	70.0%	79.5%
Total Alleles		2000	400	400	400	400	400

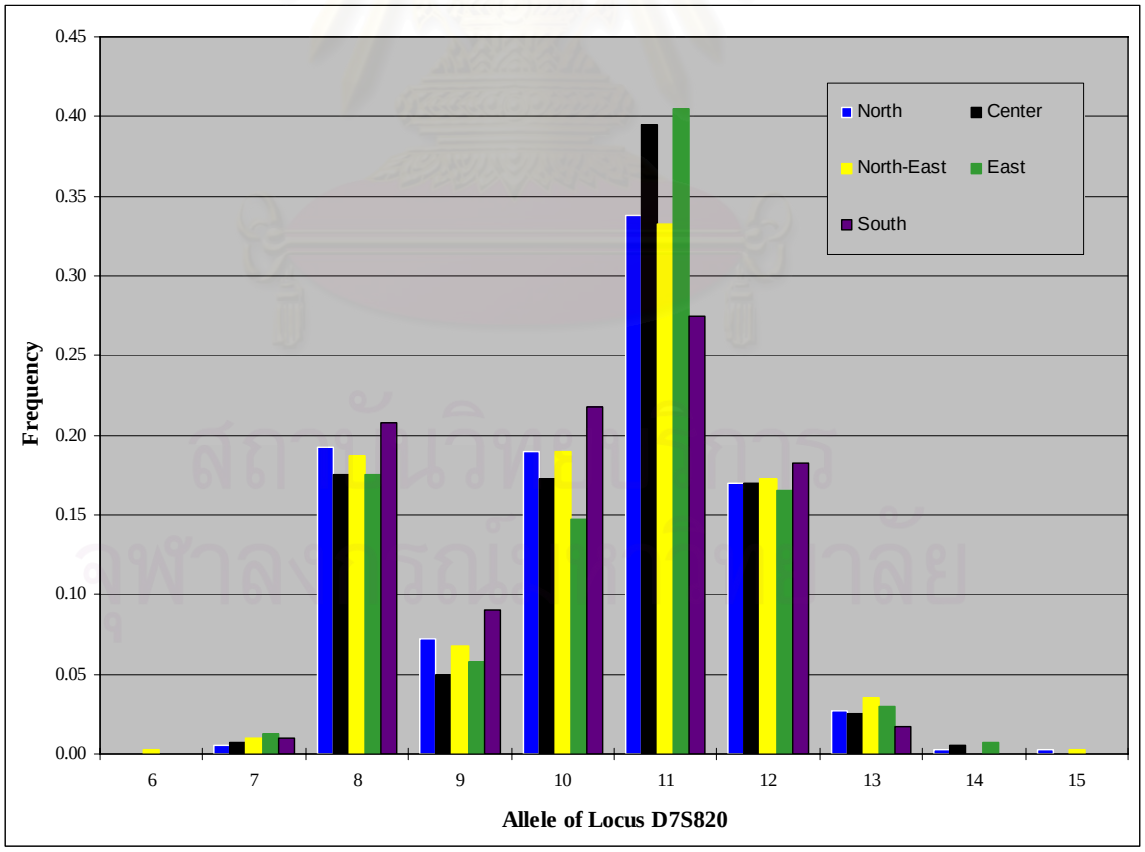

Fig. 10 The expected Allele Frequency of locus D7S820

Table. 6 Statistics for Forensic identification and Parentage studies at Locus CFS1PO

Locus CFS1PO		ALL	North	Center	North East	East	South
Forensic	Matching Probability	0.129	0.157	0.120	0.130	0.131	0.132
	Power of Discrimination	0.871	0.843	0.880	0.870	0.869	0.868
	PIC	0.67	0.64	0.68	0.68	0.66	0.66
Paternity	Power of Exclusion	0.428	0.452	0.460	0.468	0.376	0.391
	Typical Paternity Index	1.67	1.75	1.79	1.82	1.49	1.54
Allele Frequencies	Homozygotes	30.0%	28.5%	28.0%	27.5%	33.5%	32.5%
	Heterozygotes	70.0%	71.5%	72.0%	72.5%	66.5%	67.5%
Total Alleles		2000	400	400	400	400	400

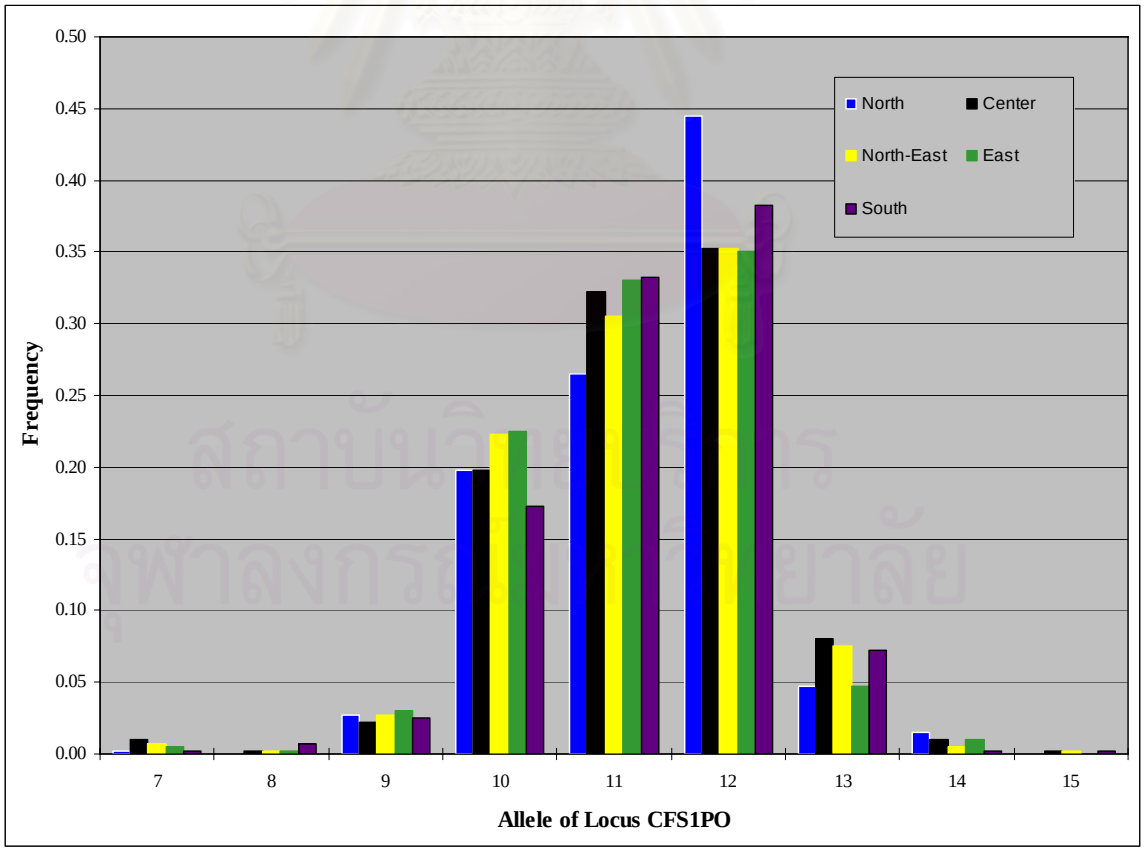

Fig. 11 The expected Allele Frequency of locus CFS1PO

Table. 7 Statistics for Forensic identification and Parentage studies at Locus D3S1358

Locus D3S1358		ALL	North	Center	North East	East	South
Forensic	Matching Probability	0.107	0.125	0.116	0.097	0.121	0.102
	Power of Discrimination	0.893	0.875	0.884	0.903	0.880	0.898
	PIC	0.70	0.70	0.70	0.72	0.68	0.71
Paternity	Power of Exclusion	0.489	0.581	0.460	0.476	0.476	0.460
	Typical Paternity Index	1.91	2.38	1.79	1.85	1.85	1.79
Allele Frequencies	Homozygotes	26.2%	21.0%	28.0%	27.0%	27.0%	28.0%
	Heterozygotes	73.8%	79.0%	72.0%	73.0%	73.0%	72.0%
Total Alleles		2000	400	400	400	400	400

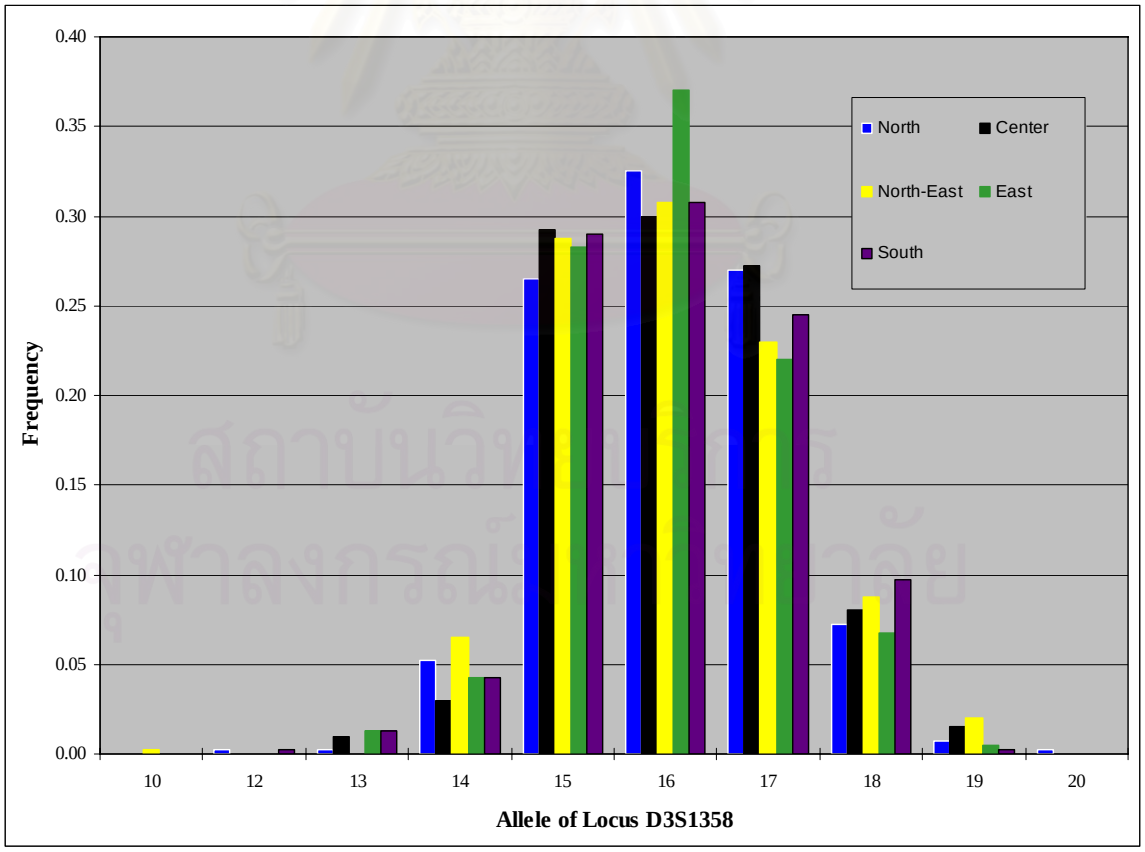

Fig. 12 The expected Allele Frequency of locus D3S1358

Table. 8 Statistics for Forensic identification and Parentage studies at Locus THO1

Locus THO1		ALL	North	Center	North East	East	South
Forensic	Matching Probability	0.109	0.124	0.112	0.125	0.117	0.091
	Power of Discrimination	0.891	0.876	0.888	0.875	0.883	0.909
	PIC	0.70	0.66	0.70	0.69	0.69	0.74
Paternity	Power of Exclusion	0.498	0.413	0.527	0.510	0.510	0.536
	Typical Paternity Index	1.95	1.61	2.08	2.00	2.00	2.13
Allele Frequencies	Homozygotes	25.7%	31.0%	24.0%	25.0%	25.0%	23.5%
	Heterozygotes	74.3%	69.0%	76.0%	75.0%	75.0%	76.5%
Total Alleles		2000	400	400	400	400	400

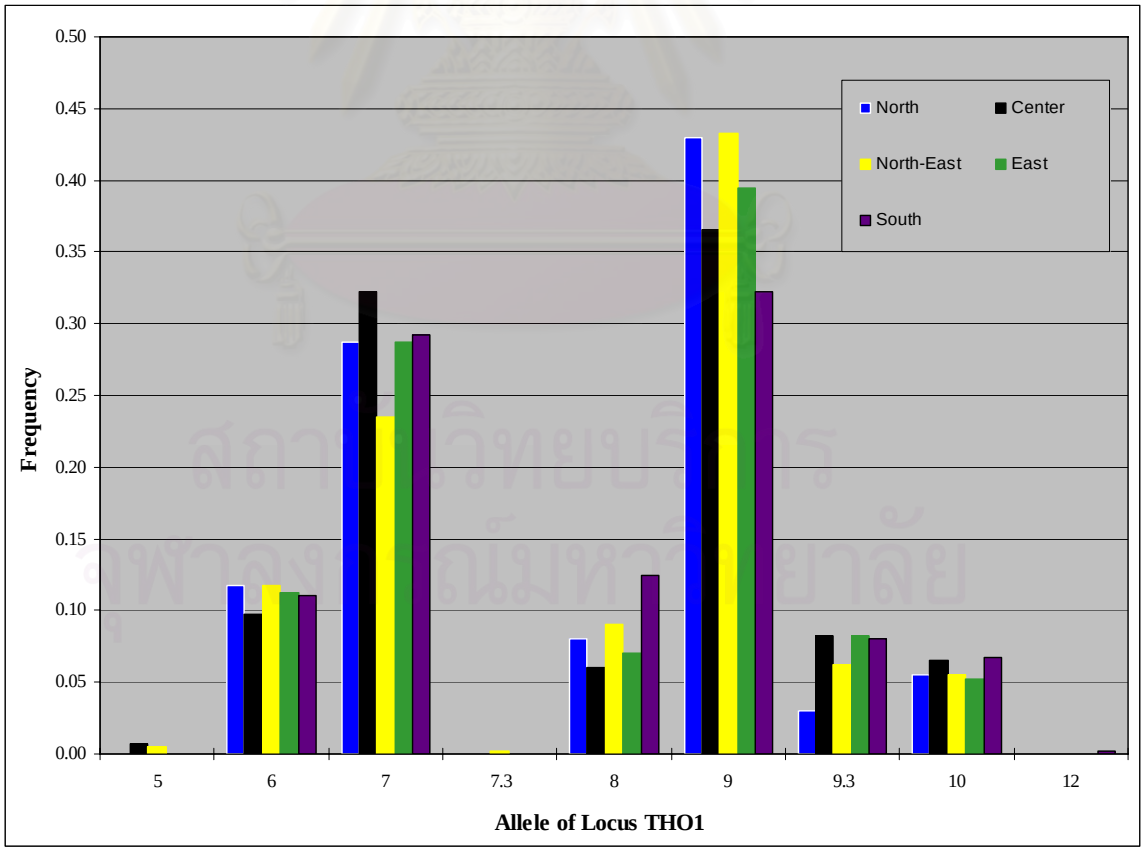

Fig. 13 The expected Allele Frequency of locus THO1

Table. 9 Statistics for Forensic identification and Parentage studies at Locus D13S317

Locus D13S317		ALL	North	Center	North East	East	South
Forensic	Matching Probability	0.075	0.072	0.081	0.082	0.081	0.084
	Power of Discrimination	0.925	0.928	0.919	0.918	0.919	0.916
	PIC	0.76	0.77	0.76	0.76	0.76	0.75
Paternity	Power of Exclusion	0.568	0.527	0.599	0.590	0.666	0.468
	Typical Paternity Index	2.30	2.08	2.50	2.44	3.03	1.82
Allele Frequencies	Homozygotes	21.7%	24.0%	20.0%	20.5%	16.5%	27.5%
	Heterozygotes	78.3%	76.0%	80.0%	79.5%	83.5%	72.5%
Total Alleles		2000	400	400	400	400	400

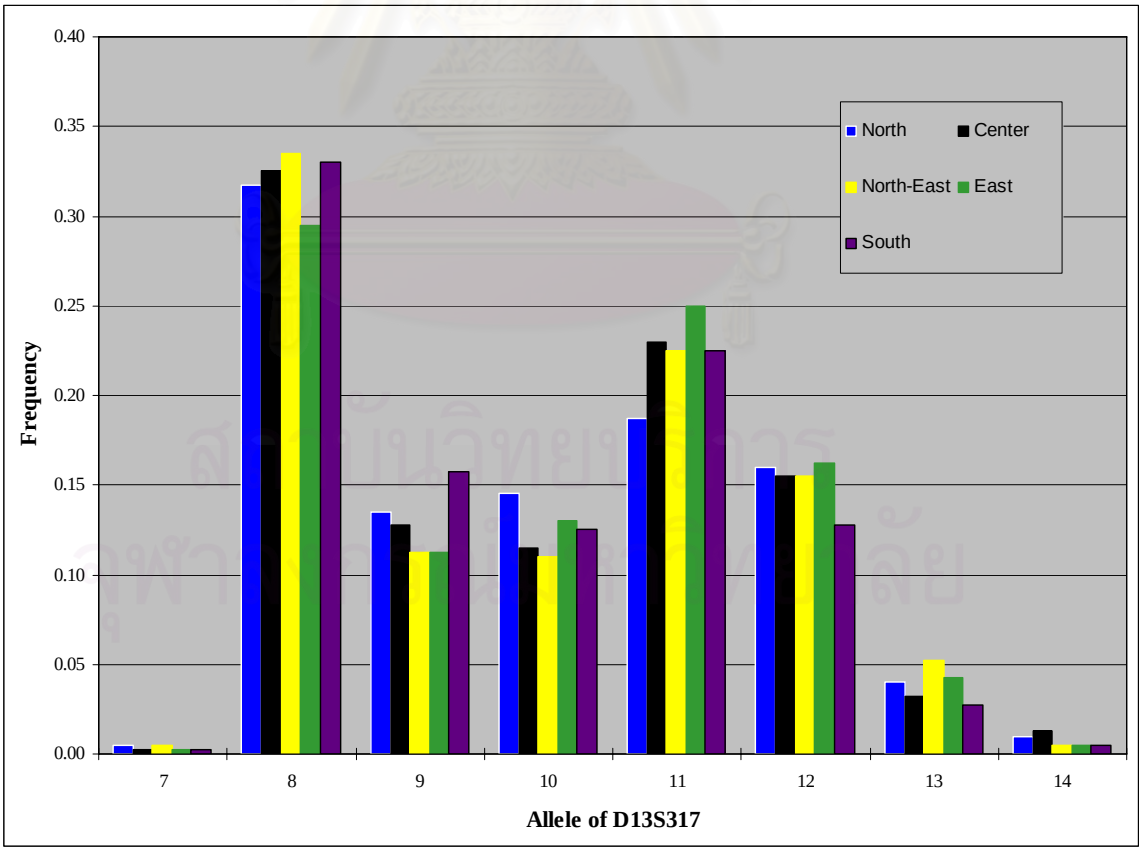

Fig. 14 The expected Allele Frequency of locus D13S317

Table. 10 Statistics for Forensic identification and Parentage studies at Locus D16S539

Locus D16S539		ALL	North	Center	North East	East	South
Forensic	Matching Probability	0.073	0.078	0.075	0.077	0.083	0.072
	Power of Discrimination	0.927	0.922	0.925	0.923	0.917	0.928
	PIC	0.76	0.75	0.76	0.76	0.77	0.77
Paternity	Power of Exclusion	0.539	0.484	0.484	0.518	0.685	0.536
	Typical Paternity Index	2.15	1.89	1.89	2.04	3.23	2.13
Allele Frequencies	Homozygotes	23.3%	26.5%	26.5%	24.5%	15.5%	23.5%
	Heterozygotes	76.7%	73.5%	73.5%	75.5%	84.5%	76.5%
Total Alleles		2000	400	400	400	400	400

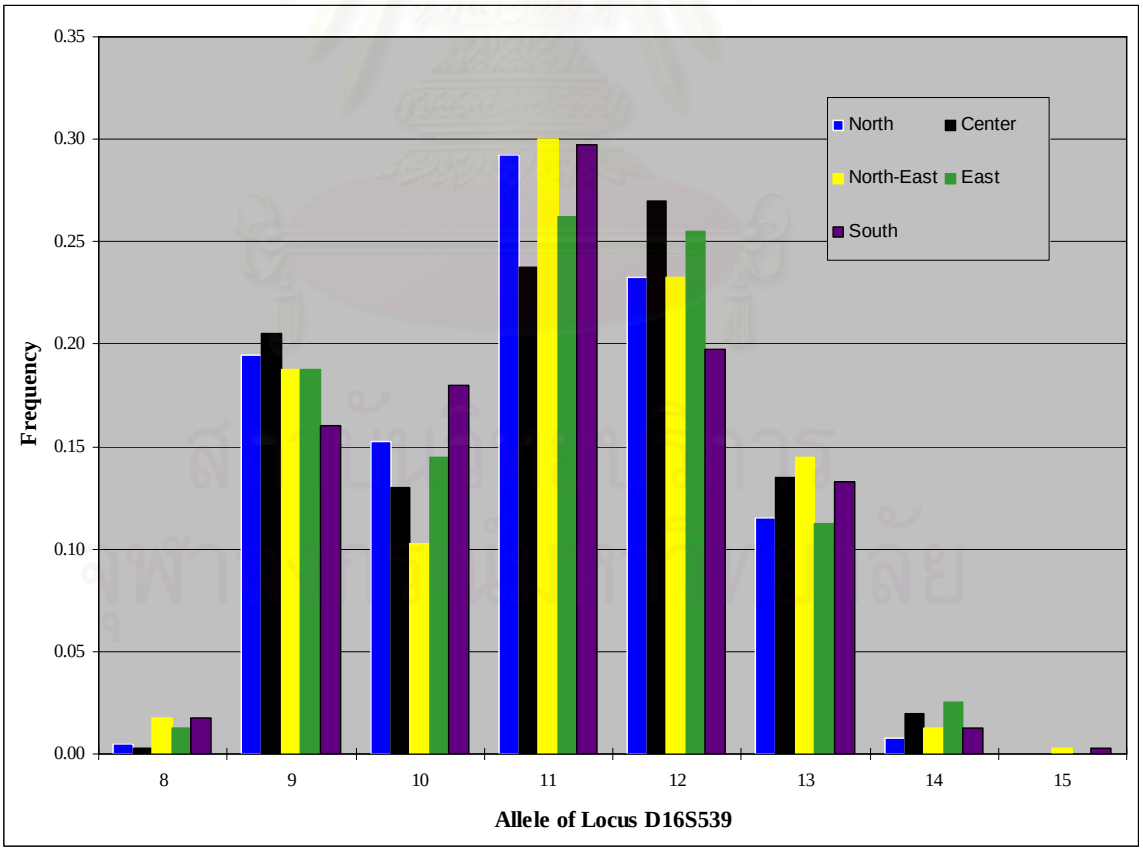

Fig. 15 The expected Allele Frequency of locus D16S539

Table. 11 Statistics for Forensic identification and Parentage studies at Locus D2S1338

Locus D2S1338		ALL	North	Center	North East	East	South
Forensic	Matching Probability	0.033	0.037	0.038	0.036	0.039	0.041
	Power of Discrimination	0.967	0.963	0.962	0.964	0.961	0.959
	PIC	0.85	0.85	0.85	0.84	0.85	0.84
Paternity	Power of Exclusion	0.705	0.675	0.755	0.666	0.785	0.646
	Typical Paternity Index	3.45	3.13	4.17	3.03	4.76	2.86
Allele Frequencies	Homozygotes	14.5%	16.0%	12.0%	16.5%	10.5%	17.5%
	Heterozygotes	85.5%	84.0%	88.0%	83.5%	89.5%	82.5%
Total Alleles		2000	400	400	400	400	400

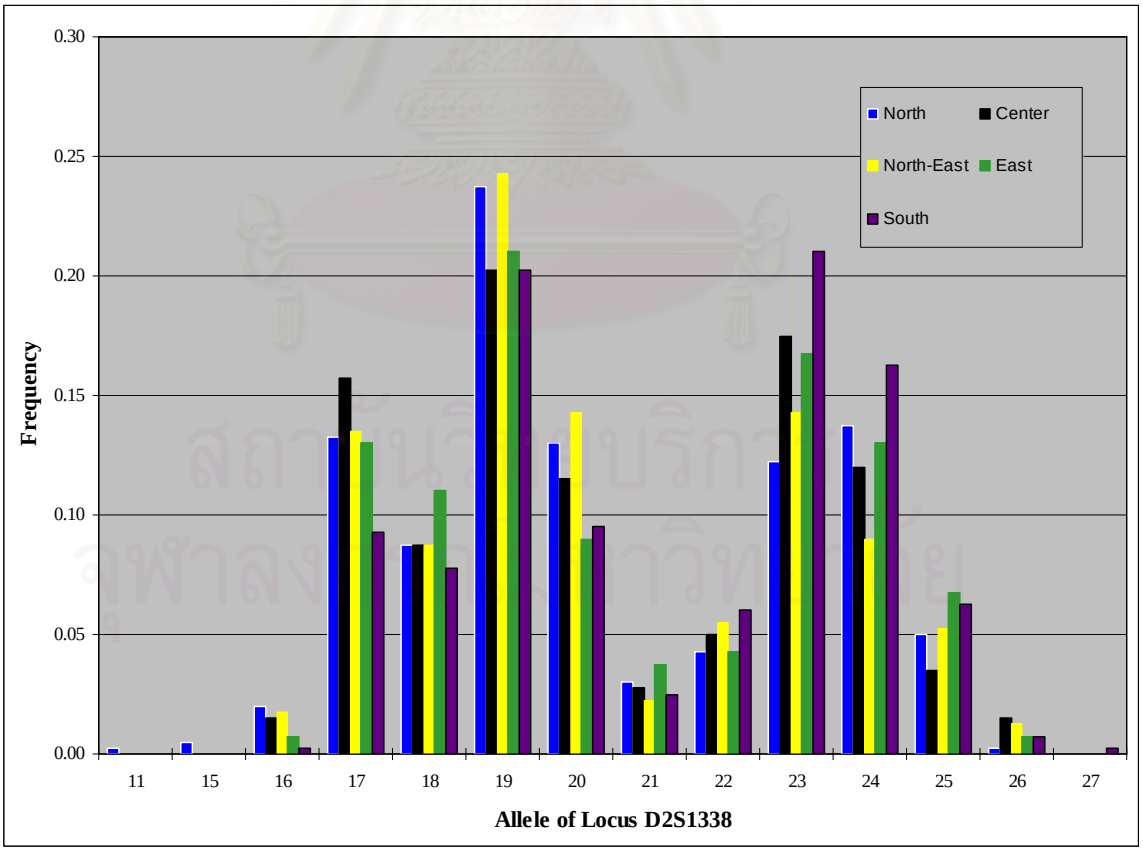

Fig. 16 The expected Allele Frequency of locus D2S1338

Table. 12 Statistics for Forensic identification and Parentage studies at Locus D19S433

Locus D19S433		ALL	North	Center	North East	East	South
Forensic	Matching Probability	0.056	0.070	0.053	0.063	0.056	0.057
	Power of Discrimination	0.944	0.930	0.947	0.937	0.944	0.943
	PIC	0.80	0.77	0.81	0.80	0.80	0.80
Paternity	Power of Exclusion	0.595	0.553	0.627	0.637	0.608	0.553
	Typical Paternity Index	2.48	2.22	2.70	2.78	2.56	2.22
Allele Frequencies	Homozygotes	20.2%	22.5%	18.5%	18.0%	19.5%	22.5%
	Heterozygotes	79.8%	77.5%	81.5%	82.0%	80.5%	77.5%
Total Alleles		2000	400	400	400	400	400

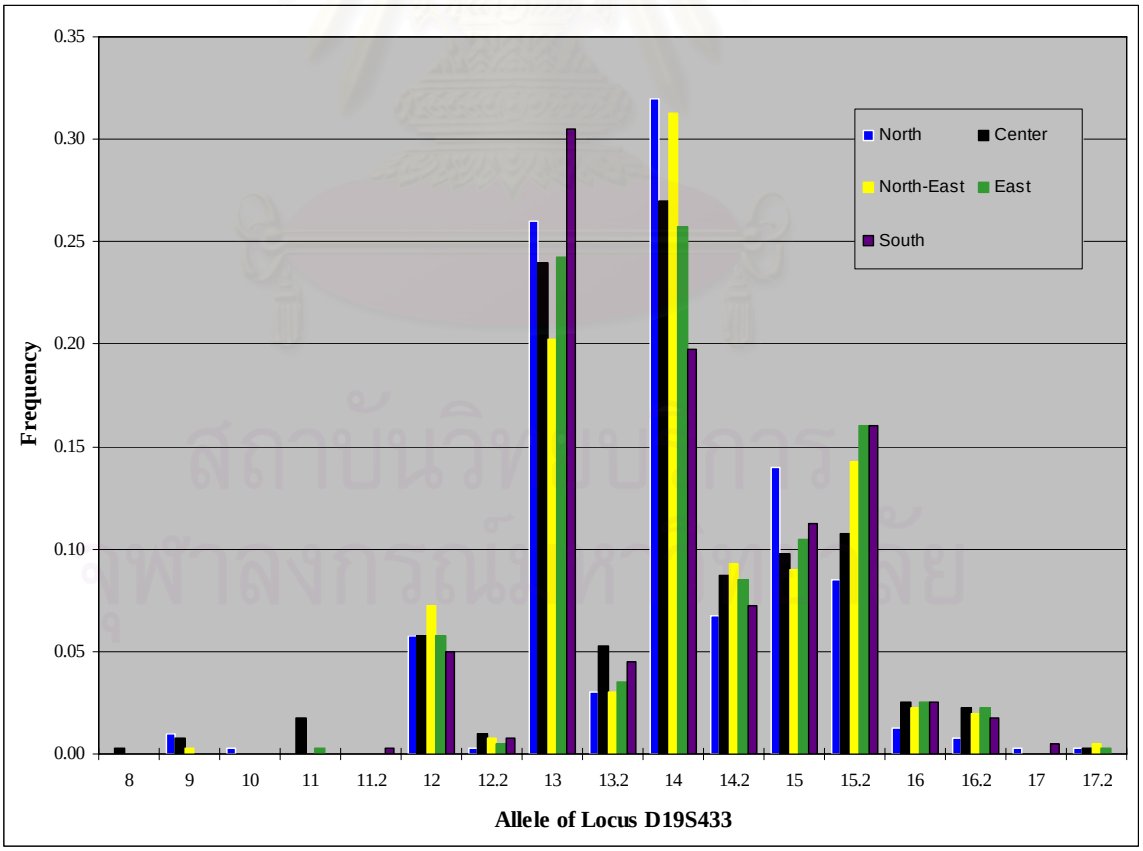

Fig. 17 The expected Allele Frequency of locus D19S433

Table. 13 Statistics for Forensic identification and Parentage studies at Locus vWA

Locus vWA		ALL	North	Center	North East	East	South
Forensic	Matching Probability	0.071	0.074	0.072	0.083	0.077	0.075
	Power of Discrimination	0.929	0.926	0.928	0.917	0.923	0.925
	PIC	0.77	0.77	0.77	0.76	0.76	0.78
Paternity	Power of Exclusion	0.599	0.581	0.646	0.685	0.536	0.553
	Typical Paternity Index	2.50	2.38	2.86	3.23	2.13	2.22
Allele Frequencies	Homozygotes	20.0%	21.0%	17.5%	15.5%	23.5%	22.5%
	Heterozygotes	80.0%	79.0%	82.5%	84.5%	76.5%	77.5%
Total Alleles		2000	400	400	400	400	400

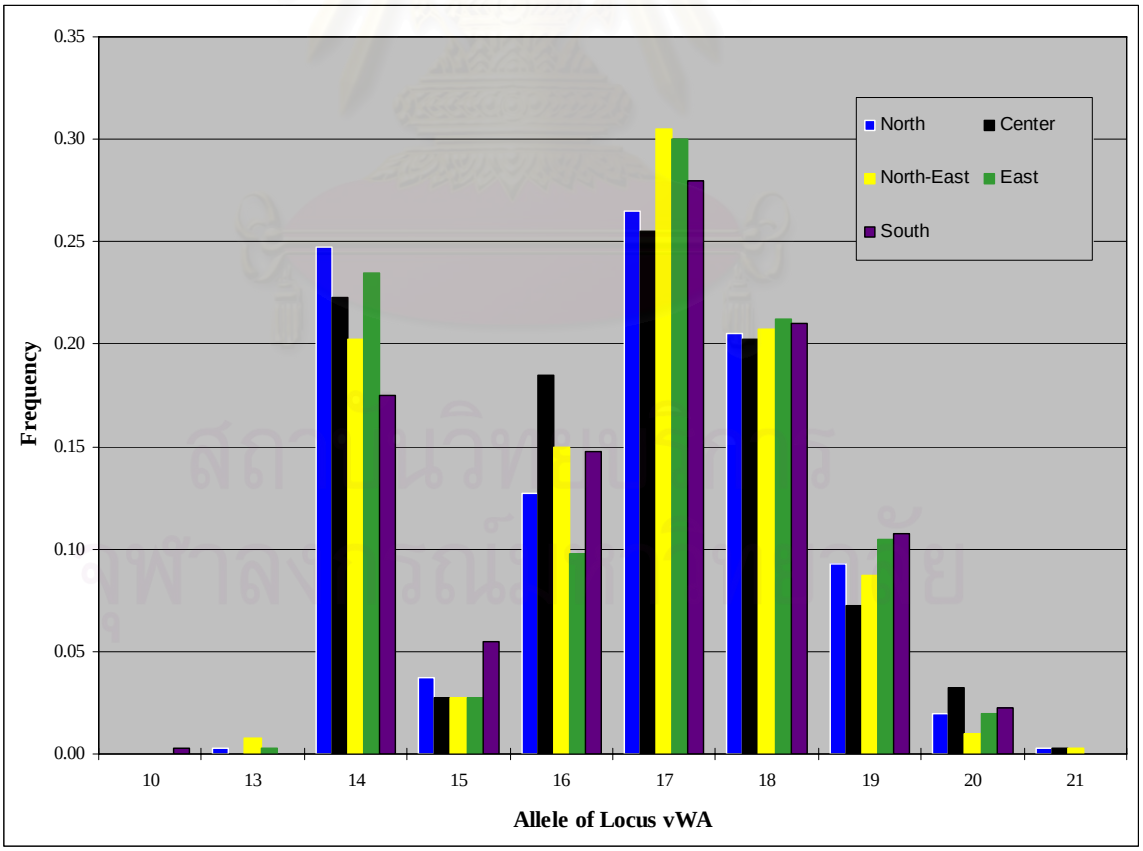

Fig. 18 The expected Allele Frequency of locus vWA

Table. 14 Statistics for Forensic identification and Parentage studies at Locus TPOX

Locus TPOX		ALL	North	Center	North East	East	South
Forensic	Matching Probability	0.206	0.193	0.230	0.203	0.212	0.208
	Power of Discrimination	0.794	0.807	0.770	0.797	0.788	0.792
	PIC	0.55	0.57	0.54	0.55	0.54	0.56
Paternity	Power of Exclusion	0.286	0.328	0.335	0.262	0.273	0.240
	Typical Paternity Index	1.24	1.35	1.37	1.18	1.20	1.12
Allele Frequencies	Homozygotes	40.4%	37.0%	36.5%	42.5%	41.5%	44.5%
	Heterozygotes	59.6%	63.0%	63.5%	57.5%	58.5%	55.5%
Total Alleles		2000	400	400	400	400	400

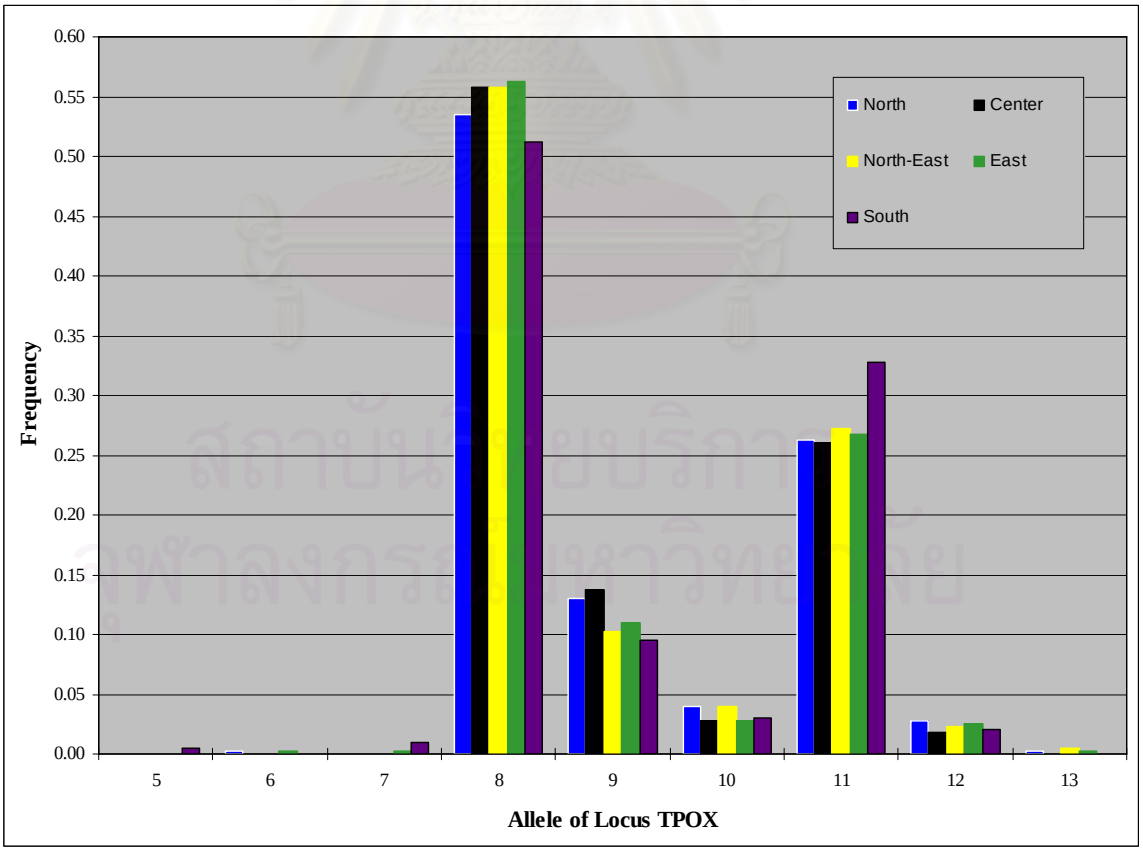

Fig. 19 The expected Allele Frequency of locus TPOX

Table. 15 Statistics for Forensic identification and Parentage studies at Locus D18S51

Locus D18S51		ALL	North	Center	North East	East	South
Forensic	Matching Probability	0.039	0.044	0.046	0.043	0.037	0.046
	Power of Discrimination	0.961	0.956	0.954	0.958	0.963	0.954
	PIC	0.83	0.83	0.83	0.83	0.84	0.83
Paternity	Power of Exclusion	0.644	0.637	0.656	0.627	0.590	0.715
	Typical Paternity Index	2.84	2.78	2.94	2.70	2.44	3.57
Allele Frequencies	Homozygotes	17.6%	18.0%	17.0%	18.5%	20.5%	14.0%
	Heterozygotes	82.4%	82.0%	83.0%	81.5%	79.5%	86.0%
Total Alleles		2000	400	400	400	400	400

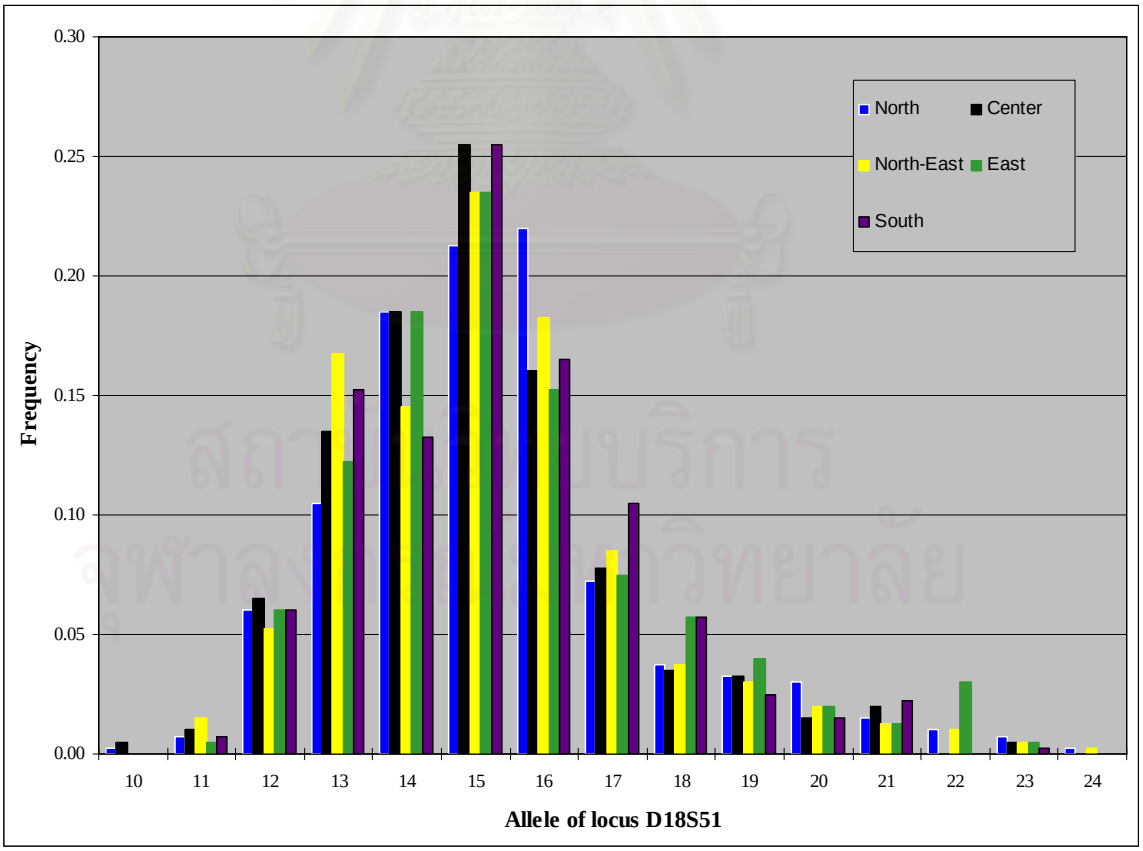

Fig. 20 The expected Allele Frequency of locus D18S51

Table. 16 Statistics for Forensic identification and Parentage studies at Locus D5S818

Locus D5S818		ALL	North	Center	North East	East	South
Forensic	Matching Probability	0.082	0.087	0.084	0.087	0.082	0.087
	Power of Discrimination	0.918	0.913	0.916	0.913	0.918	0.913
	PIC	0.75	0.74	0.74	0.74	0.76	0.74
Paternity	Power of Exclusion	0.543	0.572	0.476	0.510	0.656	0.510
	Typical Paternity Index	2.16	2.33	1.85	2.00	2.94	2.00
Allele Frequencies	Homozygotes	23.1%	21.5%	27.0%	25.0%	17.0%	25.0%
	Heterozygotes	76.9%	78.5%	73.0%	75.0%	83.0%	75.0%
Total Alleles		2000	400	400	400	400	400

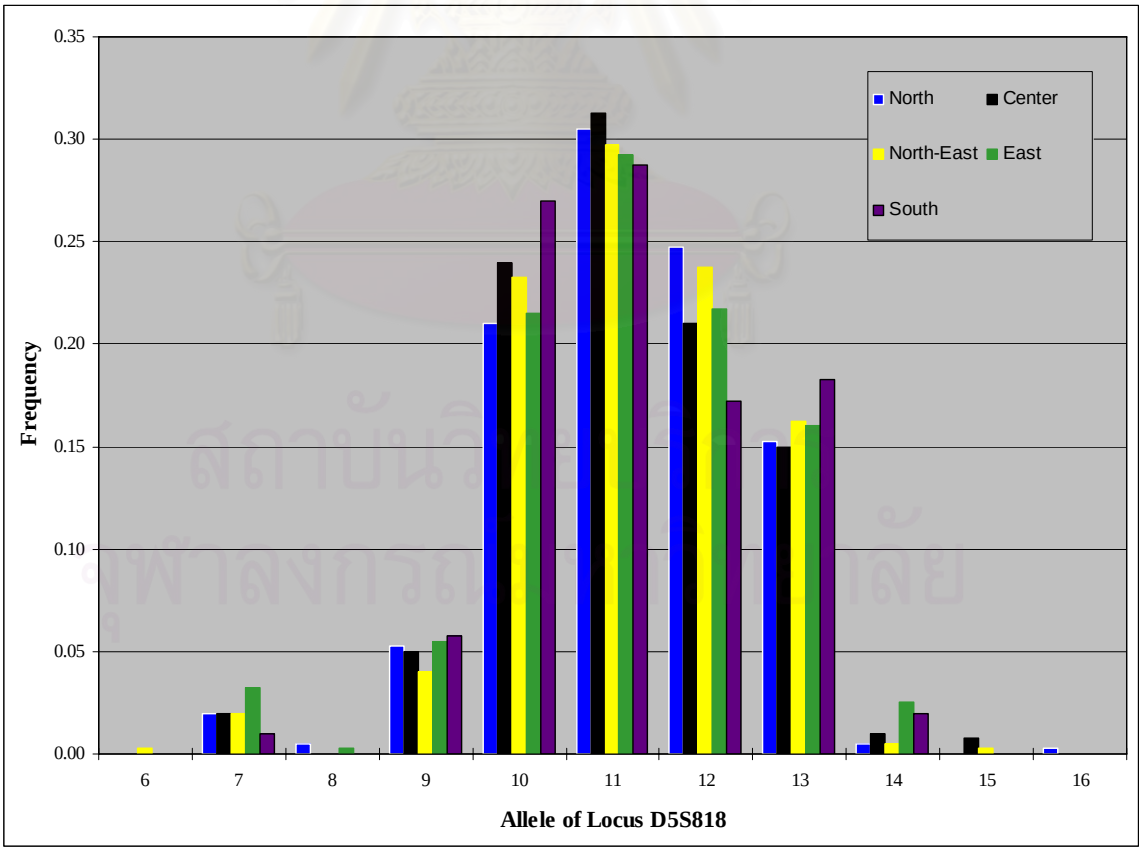

Fig. 21 The expected Allele Frequency of locus D5S818

Table. 17 Statistics for Forensic identification and Parentage studies at Locus FGA

Locus FGA		ALL	North	Center	North -East	East	South
Forensic	Matching Probability	0.029	0.030	0.035	0.031	0.034	0.033
	Power of Discrimination	0.971	0.970	0.965	0.969	0.966	0.967
	PIC	0.86	0.87	0.85	0.87	0.86	0.86
Paternity	Power of Exclusion	0.753	0.755	0.745	0.775	0.765	0.725
	Typical Paternity Index	4.13	4.17	4.00	4.55	4.35	3.70
Allele Frequencies	Homozygotes	12.1%	12.0%	12.5%	11.0%	11.5%	13.5%
	Heterozygotes	87.9%	88.0%	87.5%	89.0%	88.5%	86.5%
Total Alleles		2000	400	400	400	400	400

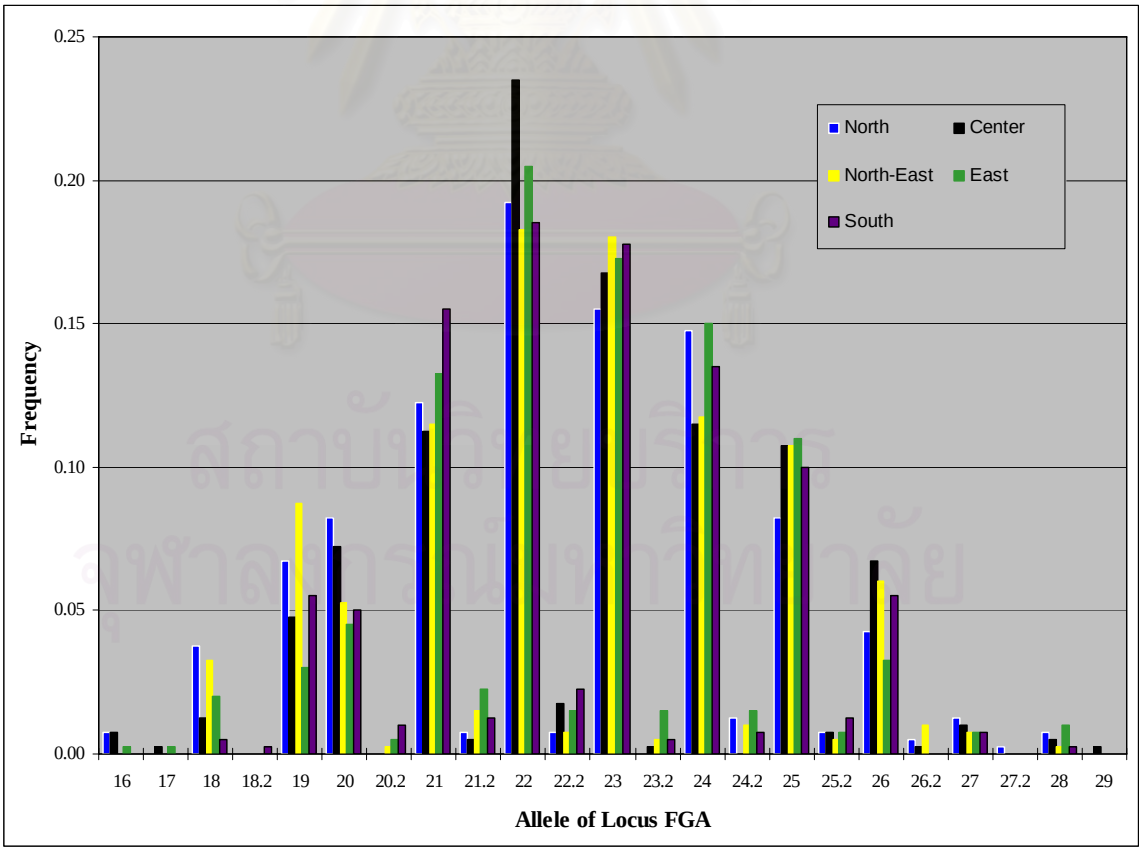

Fig. 22 The expected Allele Frequency of locus FGA

Table 18 The expected Allele Frequency of locus D8S1179

Allele	ALL	Frequency D8S1179				
		North	Center	North-East	East	South
7	0.1%	-	-	-	-	0.3%
8	0.3%	-	0.3%	0.3%	0.3%	0.5%
9	0.1%	-	-	0.5%	-	-
10	14.8%	17.3%	13.3%	15.3%	12.0%	16.3%
11	9.9%	9.0%	12.0%	11.3%	9.8%	7.5%
12	10.7%	8.0%	9.0%	10.8%	13.3%	12.5%
13	16.5%	17.8%	16.8%	16.5%	17.5%	14.0%
14	17.9%	18.0%	18.5%	18.0%	20.5%	14.3%
15	19.1%	20.3%	18.5%	17.8%	17.0%	22.0%
16	8.6%	7.5%	9.5%	9.5%	7.0%	9.3%
17	2.0%	1.5%	2.0%	0.3%	2.8%	3.3%
18	0.2%	0.8%	-	-	-	0.3%
19	0.1%	-	0.3%	-	-	-

Table. 19 The expected Allele Frequency of locus D21S11

Allele	ALL	Frequency D21S11				
		North	Center	North-East	East	South
25.2	0.1%	-	-	-	-	0.3%
26	0.1%	-	-	0.3%	-	-
27	0.3%	-	0.5%	0.3%	0.5%	-
28	6.7%	5.0%	6.8%	7.8%	6.3%	7.8%
28.2	0.1%	-	0.3%	-	-	-
29	25.5%	28.5%	23.8%	23.5%	27.3%	24.3%
29.2	0.4%	0.8%	-	0.3%	-	0.8%
30	24.1%	27.8%	25.3%	23.0%	25.0%	19.5%
30.2	3.7%	2.3%	4.3%	2.3%	4.0%	5.5%
31	8.2%	9.5%	8.3%	7.5%	6.3%	9.5%
31.2	7.2%	4.8%	8.5%	6.0%	9.0%	7.8%
32	3.9%	5.0%	5.5%	4.3%	3.0%	1.8%
32.2	14.2%	11.8%	10.0%	20.3%	11.8%	17.3%
33	0.8%	1.8%	1.3%	-	0.8%	-
33.2	4.5%	1.8%	5.0%	4.5%	6.0%	5.0%
34	0.1%	0.3%	-	-	-	-
34.2	0.4%	0.5%	0.5%	0.3%	-	0.8%
35.2	0.2%	0.3%	0.3%	-	0.3%	-
39	0.1%	0.3%	-	-	-	-

Table. 20 The expected Allele Frequency of locus D7S820

Allele	ALL	Frequency D7S820				
		North	Center	North-East	East	South
6	0.1%	-	-	0.3%	-	-
7	0.9%	0.5%	0.8%	1.0%	1.3%	1.0%
8	18.8%	19.3%	17.5%	18.8%	17.5%	20.8%
9	6.8%	7.3%	5.0%	6.8%	5.8%	9.0%
10	18.4%	19.0%	17.3%	19.0%	14.8%	21.8%
11	34.9%	33.8%	39.5%	33.3%	40.5%	27.5%
12	17.2%	17.0%	17.0%	17.3%	16.5%	18.3%
13	2.7%	2.8%	2.5%	3.5%	3.0%	1.8%
14	0.3%	0.3%	0.5%	-	0.8%	-
15	0.1%	0.3%	-	0.3%	-	-

Table. 21 The expected Allele Frequency of locus CFS1PO

Allele	ALL	Frequency CFS1PO				
		North	Center	North-East	East	South
7	0.6%	0.3%	1.0%	0.8%	0.5%	0.3%
8	0.3%	-	0.3%	0.3%	0.3%	0.8%
9	2.7%	2.8%	2.3%	2.8%	3.0%	2.5%
10	20.3%	19.8%	19.8%	22.3%	22.5%	17.3%
11	31.1%	26.5%	32.3%	30.5%	33.0%	33.3%
12	37.7%	44.5%	35.3%	35.3%	35.0%	38.3%
13	6.5%	4.8%	8.0%	7.5%	4.8%	7.3%
14	0.9%	1.5%	1.0%	0.5%	1.0%	0.3%
15	0.2%	-	0.3%	0.3%	-	0.3%

Table. 22 The expected Allele Frequency of locus D3S1358

Allele	ALL	Frequency D3S1358				
		North	Center	North-East	East	South
10	0.1%	-	-	0.3%	-	-
12	0.1%	0.3%	-	-	-	0.3%
13	0.8%	0.3%	1.0%	-	1.3%	1.3%
14	4.7%	5.3%	3.0%	6.5%	4.3%	4.3%
15	28.4%	26.5%	29.3%	28.8%	28.3%	29.0%
16	32.2%	32.5%	30.0%	30.8%	37.0%	30.8%
17	24.8%	27.0%	27.3%	23.0%	22.0%	24.5%
18	8.1%	7.3%	8.0%	8.8%	6.8%	9.8%
19	1.0%	0.8%	1.5%	2.0%	0.5%	0.3%
20	0.1%	0.3%	-	-	-	-

Table. 23 The expected Allele Frequency of locus THO1

Allele	ALL	Frequency THO1				
		North	Center	North-East	East	South
5	0.3%	-	0.8%	0.5%	-	-
6	11.1%	11.8%	9.8%	11.8%	11.3%	11.0%
7	28.5%	28.8%	32.3%	23.5%	28.8%	29.3%
7.3	0.1%	-	-	0.3%	-	-
8	8.5%	8.0%	6.0%	9.0%	7.0%	12.5%
9	38.9%	43.0%	36.5%	43.3%	39.5%	32.3%
9.3	6.8%	3.0%	8.3%	6.3%	8.3%	8.0%
10	5.9%	5.5%	6.5%	5.5%	5.3%	6.8%
12	0.1%	-	-	-	-	0.3%

Table. 24 The expected Allele Frequency of locus D13S317

Allele	ALL	Frequency D13S317				
		North	Center	North-East	East	South
7	0.4%	0.5%	0.3%	0.5%	0.3%	0.3%
8	32.1%	31.8%	32.5%	33.5%	29.5%	33.0%
9	12.9%	13.5%	12.8%	11.3%	11.3%	15.8%
10	12.5%	14.5%	11.5%	11.0%	13.0%	12.5%
11	22.4%	18.8%	23.0%	22.5%	25.0%	22.5%
12	15.2%	16.0%	15.5%	15.5%	16.3%	12.8%
13	3.9%	4.0%	3.3%	5.3%	4.3%	2.8%
14	0.8%	1.0%	1.3%	0.5%	0.5%	0.5%

Table. 25 The expected Allele Frequency of locus D16S539

Allele	ALL	Frequency D16S539				
		North	Center	North-East	East	South
8	1.1%	0.5%	0.3%	1.8%	1.3%	1.8%
9	18.7%	19.5%	20.5%	18.8%	18.8%	16.0%
10	14.2%	15.3%	13.0%	10.3%	14.5%	18.0%
11	27.8%	29.3%	23.8%	30.0%	26.3%	29.8%
12	23.8%	23.3%	27.0%	23.3%	25.5%	19.8%
13	12.8%	11.5%	13.5%	14.5%	11.3%	13.3%
14	1.6%	0.8%	2.0%	1.3%	2.5%	1.3%
15	0.1%	-	-	0.3%	-	0.3%

Table. 26 The expected Allele Frequency of locus D2S1338

Allele	ALL	Frequency D2S1338				
		North	Center	North-East	East	South
11	0.1%	0.3%	-	-	-	-
15	0.1%	0.5%	-	-	-	-
16	1.3%	2.0%	1.5%	1.8%	0.8%	0.3%
17	13.0%	13.3%	15.8%	13.5%	13.0%	9.3%
18	9.0%	8.8%	8.8%	8.8%	11.0%	7.8%
19	21.9%	23.8%	20.3%	24.3%	21.0%	20.3%
20	11.5%	13.0%	11.5%	14.3%	9.0%	9.5%
21	2.9%	3.0%	2.8%	2.3%	3.8%	2.5%
22	5.0%	4.3%	5.0%	5.5%	4.3%	6.0%
23	16.4%	12.3%	17.5%	14.3%	16.8%	21.0%
24	12.8%	13.8%	12.0%	9.0%	13.0%	16.3%
25	5.4%	5.0%	3.5%	5.3%	6.8%	6.3%
26	0.9%	0.3%	1.5%	1.3%	0.8%	0.8%
27	0.1%	-	-	-	-	0.3%

Table. 27 The expected Allele Frequency of locus D19S433

Allele	ALL	Frequency D19S433				
		North	Center	North-East	East	South
8	0.1%	-	0.3%	-	-	-
9	0.4%	1.0%	0.8%	0.3%	-	-
10	0.1%	0.3%	-	-	-	-
11	0.4%	-	1.8%	-	0.3%	-
11.2	0.1%	-	-	-	-	0.3%
12	5.9%	5.8%	5.8%	7.3%	5.8%	5.0%
12.2	0.7%	0.3%	1.0%	0.8%	0.5%	0.8%
13	25.0%	26.0%	24.0%	20.3%	24.3%	30.5%
13.2	3.9%	3.0%	5.3%	3.0%	3.5%	4.5%
14	27.2%	32.0%	27.0%	31.3%	25.8%	19.8%
14.2	8.1%	6.8%	8.8%	9.3%	8.5%	7.3%
15	10.9%	14.0%	9.8%	9.0%	10.5%	11.3%
15.2	13.1%	8.5%	10.8%	14.3%	16.0%	16.0%
16	2.2%	1.3%	2.5%	2.3%	2.5%	2.5%
16.2	1.8%	0.8%	2.3%	2.0%	2.3%	1.8%
17	0.2%	0.3%	-	-	-	0.5%
17.2	0.3%	0.3%	0.3%	0.5%	0.3%	-

Table. 28 The expected Allele Frequency of locus vWA

Allele	ALL	Frequency vWA				
		North	Center	North-East	East	South
10	0.1%	-	-	-	-	0.3%
13	0.3%	0.3%	-	0.8%	0.3%	-
14	21.7%	24.8%	22.3%	20.3%	23.5%	17.5%
15	3.5%	3.8%	2.8%	2.8%	2.8%	5.5%
16	14.2%	12.8%	18.5%	15.0%	9.8%	14.8%
17	28.1%	26.5%	25.5%	30.5%	30.0%	28.0%
18	20.8%	20.5%	20.3%	20.8%	21.3%	21.0%
19	9.3%	9.3%	7.3%	8.8%	10.5%	10.8%
20	2.1%	2.0%	3.3%	1.0%	2.0%	2.3%
21	0.2%	0.3%	0.3%	0.3%	-	-

Table. 29 The expected Allele Frequency of locus TPOX

Allele	ALL	Frequency TPOX				
		North	Center	North-East	East	South
5	0.1%	-	-	-	-	0.5%
6	0.1%	0.3%	-	-	0.3%	-
7	0.3%	--	-	-	0.3%	1.0%
8	54.5%	53.5%	55.8%	55.8%	56.3%	51.3%
9	11.5%	13.0%	13.8%	10.3%	11.0%	9.5%
10	3.3%	4.0%	2.8%	4.0%	2.8%	3.0%
11	27.8%	26.3%	26.0%	27.3%	26.8%	32.8%
12	2.3%	2.8%	1.8%	2.3%	2.5%	2.0%
13	0.2%	0.3%	-	0.5%	0.3%	-

Table. 30 The expected Allele Frequency of locus D18S51

Allele	ALL	Frequency D18S51				
		North	Center	North-East	East	South
10	0.2%	0.3%	0.5%	-	-	-
11	0.9%	0.8%	1.0%	1.5%	0.5%	0.8%
12	6.0%	6.0%	6.5%	5.3%	6.0%	6.0%
13	13.7%	10.5%	13.5%	16.8%	12.3%	15.3%
14	16.7%	18.5%	18.5%	14.5%	18.5%	13.3%
15	23.9%	21.3%	25.5%	23.5%	23.5%	25.5%
16	17.6%	22.0%	16.0%	18.3%	15.3%	16.5%
17	8.3%	7.3%	7.8%	8.5%	7.5%	10.5%
18	4.5%	3.8%	3.5%	3.8%	5.8%	5.8%
19	3.2%	3.3%	3.3%	3.0%	4.0%	2.5%
20	2.0%	3.0%	1.5%	2.0%	2.0%	1.5%
21	1.7%	1.5%	2.0%	1.3%	1.3%	2.3%
22	1.0%	1.0%	-	1.0%	3.0%	-
23	0.5%	0.8%	0.5%	0.5%	0.5%	0.3%
24	0.1%	0.3%	-	0.3%	-	-

Table. 31 The expected Allele Frequency of locus D5S818

Allele	ALL	Frequency D5S818				
		North	Center	North-East	East	South
6	0.1%	-	-	0.3%	-	-
7	2.1%	2.0%	2.0%	2.0%	3.3%	1.0%
8	0.2%	0.5%	-	-	0.3%	-
9	5.1%	5.3%	5.0%	4.0%	5.5%	5.8%
10	23.4%	21.0%	24.0%	23.3%	21.5%	27.0%
11	29.9%	30.5%	31.3%	29.8%	29.3%	28.8%
12	21.7%	24.8%	21.0%	23.8%	21.8%	17.3%
13	16.2%	15.3%	15.0%	16.3%	16.0%	18.3%
14	1.3%	0.5%	1.0%	0.5%	2.5%	2.0%
15	0.2%	-	0.8%	0.3%	-	-
16	0.1%	0.3%	-	-	-	-

Table. 32 The expected Allele Frequency of locus FGA

Allele	ALL	Frequency FGA				
		North	Center	North-East	East	South
16	0.4%	0.8%	0.8%	-	0.3%	-
17	0.1%	-	0.3%	-	0.3%	-
18	2.2%	3.8%	1.3%	3.3%	2.0%	0.5%
18.2	0.1%	-	-	-	-	0.3%
19	5.8%	6.8%	4.8%	8.8%	3.0%	5.5%
20	6.1%	8.3%	7.3%	5.3%	4.5%	5.0%
20.2	0.4%	-	-	0.3%	0.5%	1.0%
21	12.8%	12.3%	11.3%	11.5%	13.3%	15.5%
21.2	1.3%	0.8%	0.5%	1.5%	2.3%	1.3%
22	20.0%	19.3%	23.5%	18.3%	20.5%	18.5%
22.2	1.4%	0.8%	1.8%	0.8%	1.5%	2.3%
23	17.1%	15.5%	16.8%	18.0%	17.3%	17.8%
23.2	0.6%	-	0.3%	0.5%	1.5%	0.5%
24	13.3%	14.8%	11.5%	11.8%	15.0%	13.5%
24.2	0.9%	1.3%	-	1.0%	1.5%	0.8%
25	10.2%	8.3%	10.8%	10.8%	11.0%	10.0%
25.2	0.8%	0.8%	0.8%	0.5%	0.8%	1.3%
26	5.2%	4.3%	6.8%	6.0%	3.3%	5.5%
26.2	0.4%	0.5%	0.3%	1.0%	-	-
27	0.9%	1.3%	1.0%	0.8%	0.8%	0.8%
27.2	0.1%	0.3%	-	-	-	-
28	0.6%	0.8%	0.5%	0.3%	1.0%	0.3%
29	0.1%	-	0.3%	-	-	0.0%

Table 32.These Chi-square tests show no significant difference of heterozygosity of all 15 loci.

Locus	Chi-Square	df	Asymp.Sig
D8S1179	1.85	4	0.763
D21S11	4.415	4	0.348
D7S820	7.737	4	0.074
CFS1PO	2.952	4	0.566
D3S1358	3.6	4	0.463
THO1	3.854	4	0.426
D13S317	8.275	4	0.082
D16S539	9.266	4	0.055
D2S1338	6.05	4	0.195
D19S433	2.333	4	0.675
vWA	5.75	4	0.219
TPOX	4.087	4	0.394
D18S51	3.131	4	0.536
D5S818	7.003	4	0.136
FGA	0.696	4	0.952

CHAPTER V

DISSCUSSION AND CONCLUSION

DNA Fingerprint Sequence data base From genetic material study, all 16 short tandem repeats are D8S1179, D21S11, D7S820, CSF1P0, D3S1358, THO1, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, Amelogenin, D5S818 and FGA

First, the extraction of DNA from buccal swab by chelex method was done. Because low cost and time wasted, extracted DNA from this method is appropriate for routine laboratory testing. Next step is duplication of DNA and analyzing by Automate DNA sequencer .

The result shows that difference of 1000 subjects' DNA fingerprint in all 15 loci. And the appropriate amount for duplication of DNA is 0.12 µl. The result can be calculated for statistic, applied for person identification and used in Forensic Medicine.

Probability Matching (PM) is chance of matching between DNA of non relative person and subject's DNA. The result from 1000 sample from AmpFlSTR® Identifiler™ PCR Amplification Kit shows 3.49×10^{-16}. The results of northern, north east, east, central, and southern regions are 1.73×10^{-15}, 1.04×10^{-15}, 6.06×10^{-16}, 8.31×10^{-16}, and 9.83×10^{-16} respectively. Hence, chance of AmpFlSTR® Identifiler™ PCR Amplification Kit identify identical person is 1 in 2.87×10^{17}.Compare with African American is 1 in 7.64×10^{17} Caucasian is 1 in 2.0×10^{17} Hispanic is 1 in 1.31×10^{17} and Native American is 1 in 2.76×10^{16}

Power of Discrimination (PD) is opposite value of probability matching which mean that chance of difference between DNA of non relative person and subject's DNA. PD can be calculated by 1-PM. The result from 1000 sample from AmpFlSTR® Identifiler™ PCR Amplification Kit shows PD in range 0.794 – 0.971 by results show that locus "FGA" has the highest Power of Discrimination; 0.971.and locus "TPOX" has the lowest Power of Discrimination; 0.794. The results of southern, north-east, central, and

east, northern by results show that locus “FGA” has the highest Power of Discrimination .and locus “TPOX” has the lowest Power of Discrimination.

Power of Exclusion (PE) or paternity exclusion is ability to exclude non paternal person. The result from 1000 sample from AmpFlSTR® Identifiler™ PCR Amplification Kit shows. Power of Exclusion in range 0.286 – 0.753 by results show that locus “FGA” has the highest Power of Exclusion; 0.753.and locus “TPOX” has the lowest Power of Exclusion; 0.286 . The results of north east, east, northern, southern, central regions are by results show that locus “FGA” has the highest Power of Exclusion .and locus “TPOX” has the lowest Power of Exclusion

Paternity index (PI) shows probability of being father of suspected person compare with other. The result from 1000 sample from AmpFlSTR® Identifiler™ PCR Amplification Kit shows. Paternity index in range 0.413 -1.24 by results show that locus “FGA” has the highest Paternity index; 1.24.and locus “TPOX” has the lowest Paternity index; 0.413 . The results of north east, east, central, southern, northern regions are. By results show that locus “FGA” has the highest Paternity index .and locus “TPOX” has the lowest Paternity index

Polymorphism information content (PIC) shows polymorphism of STR loci. It shows how many alleles and difference of frequency of each allele

The study at locus D8S1179 Centre found allele 19, Southern found allele 7 and Northern-Eastern found allele 9 difference form another area. In Thai population found STR Locus D8S1179 7-19 allele Difference form Malaysian Found 9-18 allele, Japan found 9-17 alleles; Poland found 9-16 alleles.

The study at locus D21S11 Centre found allele 28.2, Southern found allele 25.2 Northern-Eastern found allele 26, and Northern found allele 34 and 49 difference form another area. In Thai population found STR Locus D8S1179 25.2-36,39 allele Difference form Malaysian Found 23.2-34.2 allele, Japan found 28-35.2 allele, Poland found 26-34.2 allele.

The study at locus D7S820 Northern-Eastern found allele 6 not found allele 14 difference form another area. In Thai population found STR Locus D7S820 6-15 allele

Difference form Malaysian Found 5-14 allele, Japan found 8-14 allele, Poland found 7-13 allele.

The study at locus CFS1PO Northern not found allele 8 and 15 difference form another area. In Thai population found STR Locus CFS1PO 7-15 allele Difference form Malaysian Found 8-15 allele, Japan found 7-17 allele, Poland found 7, 9-15 allele.

The study at locus D3S1358 Northern-Eastern found allele 10 not found allele 12, 13 difference form another area. In Thai population found STR Locus D3S1358 10,12-20 allele Difference form Malaysian Found 12-20 allele, Japan found 12,14-20 allele, Poland found 14-19 allele.

The study at locus THO1 Northern-Eastern found allele 5, 7.3 and Southern found allele 12 difference form another area. In Thai population found STR Locus THO1 5-10, 12 allele Difference form Malaysian Found 5-11 allele, Japan found 6-10 allele, Poland found 6-10 allele.

The study at locus D13S317 In Thai population found STR Locus D13S317 7-14 allele Difference form Malaysian Found 7-15 allele, Japan found 7-14 allele, Poland found 8-15 allele.

The study at locus D16S539 Southern and Northern-Eastern found allele 15 difference form another area . In Thai population found STR Locus D16S539 8-15 allele Difference form Malaysian Found 8-14 allele, Japan found 8-15 allele, Poland found 10,16-268-14,16,16.2 allele

The study at locus D2S1338 Northern found allele 11 and 15, Southern found allele 27 difference form another area . In Thai population found STR Locus D2S1338 11-27 allele Difference form Malaysian Found 15-26 allele, Japan found 15-28 allele, Poland found 10,16-26 allele

The study at locus D19S433 Northern-Eastern found allele 9, Eastern found allele 11, Southern found allele 11.2 and not found allele 17.2 difference form another area .In Thai population found STR Locus D19S433 8-17.2 allele Difference form Malaysian Found 9,11-17 allele, Japan found 10-16.2,17.2 allele, Poland found 12-17,18.2 allele

The study at locus vWA Southern found allele 10 and not found allele 13,21 difference form another area .In Thai population found STR Locus vWA 10-21 allele. Difference form Malaysianian Found 13-20 allele, Japan found 13-20 allele, Poland found 14-20 allele

The study at locus TPOX Southern found allele 5 not found allele 6,13 ;Northern not found allele 5, 7 ; Northern-Eastern not found 5-7 difference form another area .In Thai population found STR Locus TPOX 5-13 allele. Difference form Malaysian Found 7-12 allele, Japan found 8-14 allele, Poland found 8-13 allele

The study at locus D18S51 Center not found allele 22,24 ; Northern-East not found 10 ; Eastern not found allele 10, 24; Southern not found allele 10,22,24 difference form another area .In Thai population found STR Locus D18S51 5-13 allele. Difference form Malaysian Found 7-12 allele, Japan found 8-14 allele, Poland found 8-13 allele

The study at D5S818 Northern found allele 8, 16 not found allele 6, 15 ;Northern-East found 6 not found 8, 16 ; Southern not found 6,8,15,16 difference form another area .In Thai population found STR Locus D18S51 6-16 allele. Difference form Malaysian Found 7-17 allele, Japan found 7-15 allele, and Poland found 9-13 allele

The study at FGA found these locus is best variation for person identification In Thai population found STR Locus D18S51 16-29 allele. Difference form Malaysian Found 17-28 allele, Japan found 17-28 allele, and Poland found 16-26 allele

This study show how do allele frequencies of Thai population be and also allele frequency of population in each region of Thai people. Probability Matching are calculated by information of all 15 loci. If the samples are represent Thai population. They tell us that they can be applied for person identification purpose in all region of Thailand. It is the most applicable for the southern people. In the study of 15 loci, Power of distribution, Power of exclusion and Paternity index show that the locus “FGA” is the best locus for person identification. The locus “TPOX” is the least appropriate locus for this purpose among all of the applicable 15 loci. By testing with Chi-square test, allele frequencies, of all 15 loci, of each population show no statistically significant difference, 95% confident interval. It means that they have share feature. Some environmental effects that lead to some minor difference of each population can be migration, variety of

nationality and marriage of people form difference region. From all these in formations, they can be concluded that these 15 loci can be a good representation for person identification of Thai people.

The population of this study was random chosen and was chosen from non-relative person. The number of population in this study, 1000 persons, is the highest number since studies of allele frequency of Thai people has been established. And this study has been done recently so this information should be the most up to date information for now. By these points this studied can be the reliable one that can be applied for many useful purposes.

The utility of this method is the same as selected for some another studies. The identifier kit has abilities in person identification by its 15 loci and the other one locus for sex identification. As discussed above using of this kit to identify all loci is the reliable method. Though each loci has difference ability for identification.

The study's result of allele frequency in Thai population has no significant difference. Compare with other former study in other 3 races in Malaysiania. They are Malaysianian, Indian and Chinese. The study also shows no significant difference of allele frequency as the result of Thai study. The studies of allele frequency in European show no significant difference along the country in Europe. But the European studies show some differences from Asian studies. It may cause by the kit was developed for the European people. When it is used with Asian people, it may cause some difference results.

REFERENCES

1. สถาบันนิติวิทยาศาสตร์. การชันสูตรพลิกศพและการตรวจที่เกิดเหตุสำหรับผู้ช่วยชันสูตรพลิกศพ. พิมพ์ครั้งที่ 1. กรุงเทพมหานคร: โรงพิมพ์คุรุสภาลาดพร้าว, 2546.
2. สำนักงานตำรวจแห่งชาติ. ประมวลระเบียบการตำรวจเกี่ยวกับคดี. กรุงเทพมหานคร: วิศิษฎ์สรอรรถการพิมพ์, 2545.
3. พิชัย นิลทองคำ. ประมวลกฎหมายวิธีพิจารณาความอาญา. กรุงเทพมหานคร: โรงพิมพ์เรือนแก้วการพิมพ์, 2535.
4. ทัศนะ สุวรรณจูฑะ. นิติเวชศาสตร์หลักปฏิบัติและวิเคราะห์ปัญหา. กรุงเทพมหานคร: บพิธการพิมพ์, 2536.
5. พงศกรณ์ ชูเวช,พ.ต.ต. การพิสูจน์หลักฐาน. กรุงเทพมหานคร: สำนักพิมพ์นิติบรรณการ, 2531.
6. วิชัย บุญแสง และคนอื่นๆ. ลายพิมพ์ดีเอ็นเอ ... จากสารพันธุกรรมสู่เทคโนโลยีพิสูจน์บุคคล. พิมพ์ครั้งที่ 1. กรุงเทพมหานคร: ฝ่ายนิเทศสัมพันธ์ สำนักงานพัฒนาวิทยาศาสตร์ และเทคโนโลยีแห่งชาติ, 2541.
7. วิชัย บุญแสง และ นุสรา สิทธิดิลกรัตน์. เอกสารการประชุมวิชาการเชิงปฏิบัติการ เรื่อง PCR กับการสร้างลายพิมพ์ ดีเอ็นเอ. (ม.ป.ท.): 2540.
8. Boerwinkle, E.; Xiong, W. Rapid Typing of Tandemly Repeated Hypervariable Loci by the Polymerase Chain Reaction. Proc .Natl. Acad. Sci. 86: 212-216
9. วิสุทธิ์ ใบไม้. พันธุศาสตร์ฉบับปรับปรุงใหม่. พิมพ์ครั้งที่ 1. กรุงเทพมหานคร: เอ็นพีซับพลายพริ้นติ้ง, 2538.

10. Krawczak, M.,and Schmidtke, J. DNA Fingerprinting. Oxford: BIOS Scientific Publishers.,1994.

11. Chakaborty, J.T. and Jeffreys, A.J. DNA Fingerprinting. Switzerland.:State of Science .Birkhauser Verlag.Basel, 1993.

12. Gill, P.; Jeffreys, A.J.; Werrelt. Forensic Application of DNA Fingerprints. Nature 316: 69-76.

13. Jeffreys, A.J;Wilson, V. and Thein, S.L. Hypervariable Minisatellite Regions in Human DNA. Nature 314: 67-73.

14. Huang, N.U.; Schumn, J. and Budowle, B. Chinese Population Data on three Tetrameric Short Tandem Repeat Loci –HUMTHO1,TPOX and CSF1PO – Derved using Multiplex PCR and Manual Typing. Forensic Science International. 71 (1995): 131-136.

15. Tautz, D. Hypervariability of simple sequences as a general source for Polymorphic DNA markers. Nucleic Acids Research 17 (1989): 6463-6471

16. วิชัย บุญแสง. การใช้ดีเอ็นเอในการตรวจพิสูจน์เอกลักษณ์. วารสารมหาวิทยาลัยนเรศวร 3 (กรกฎาคม –ธันวาคม 2538): 5-10.

17. Litt, M.and ;Luty, J.A. A hypervariable microsatellite revealed by in vitro amplification of a dinecleotide repeat within the cardiac muscle actin gene. American Journal of Human Genetics 44 (1989): 397-401

18. Weber, J.;L. and May, P.E. Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. American Journal of Human Genetics 44 (1989): 388-396.

19. Edwards, A.; Hammond, H.A.; Jin, L Caskey,; Chakraborty, R. Genetic variation of five trimeric and tetrameric tandem repeat loci in four human population groups. Genomics 12 (1992): 241-253.

20. วิชัย บุญแสง. การสร้างและการประยุกต์ใช้ลายพิมพ์ดีเอ็นเอในคน. วารสารมหาวิทยาลัยมหิดล 3 (กรกฎาคม – กันยายน 2539): 139-144.

21. Cabrero, C.; Diez, A.; Valverde, E. and Caeeacedo, A.J. Allele Frequency Distribution of Four PCR Amplified Loci in the Spanish population. Forensic Science International.71: (1995).153-164.

22. Hashiyada, M.; Itakura, J.; Polymorphism of 17 STRs by multiplex analysis in Japanese Population. Forensic Science International. 133: (1995).250-253.

23. Applied Biosystems. AmpFISTRR Identifilerk PCR Amplification Kit User's Manual, Foster City, 2001.

24. Chantratita,W. ,et.al. Thai population data on nine tetrameric STR loci D3S1358 ,vWA, FGA, TH01, TPOX, CSF1PO,D5S818, D13S317 and D7S820. Forensic Science International 115: (2001) 113-115.

25. Miguel, Angel, Chiurillo, et.al. Genetic profiling of a central Venezuelan population using 15 STR markers that may be of forensic importance. Forensic Science International 136 : (2003) 99–101.

26. Lay ,Hong.; Seah, N.; Hithaya Jeevan . STR Data for the AmpFlSTR Identifiler loci in three ethnic groups (Malay, Chinese, Indian) of the Malaysian population. Forensic Science International.138: (2003).134-137.

27. Anna, Barbaro,.et.al. Population genetic study of 15 STRs loci usingAmpF/STR Identifilerk kit. International Congress Series 1239 (2003) 259–266.

28. Yoo-Li Kim; Ji-Yeon Hwang; Yoo-Jin Kim. Allele frequencies of 15 STR loci

using AmpF/STR Identifiler kit in a Korean population. Forensic Science International.136: (2003) .92-95.

29. Witold Pepinski et.al. Genetic data on 15 STRs in a population sample of religious minority of Old Believers residing in the northeastern Poland. Forensic Science International 139 : (2004) 24-26.

30. Morales, J.A.; Monterrosa, J.C. Population genetic data from El Salvador (Central America) using AmpFlSTRR IdentifilerR PCR Amplification Kit : International Congress Series 1261: (2004) 223– 225.

31. Kubat, M. et.al. Population genetics of the 15 AmpFl STR Identifiler loci in Kosovo Albanians. Int J Legal Med 118: (2004) 115–118.

32. Santos, M.V. et.al. Population genetic data for the STR loci using the AmpFlSTRR Identifilerk Kit in Bahia, Brazil. International Congress Series 1261: (2004) 219– 222.

APPENDICES

APPENDIX A

Glossary

Allele one of two or more alternative forms of a gene

Allele frequency the proportion of a particular allele among the Chromosomes carried by individuals in a population

Amplicons PCR products

Basepair two complementary nucleotides held together by hydrogen bonds; basepairing occurs between A and T and between G and C

Controls tests performed in parallel with experimental samples and designed to demonstrate that a procedure worked correctly

Degradation the breaking down of DNA by chemical or physical means

Denaturing the process of unfolding of the complementary double strands of DNA to form single strands

DNA database a collection of DNA typing profiles of selected or randomly chosen individuals

DNA polymerase an enzyme that catalyzes the synthesis of double-stranded DNA

Electrophoresis a technique in which different molecules are separated by heir rate of movement in an electric field

Gel semisolid matrix (usually agarose or acrylamide) used in electrophoresis to separate molecules

Genome the total genetic makeup of an organism

Genotype the genetic makeup of an organism, as distinguished from its physical appearance or phenotype

Heterozygote a diploid organism that carries different alleles at one or more genetic loci on its homologous chromosomes

Heterozygous having different alleles at a particular locus; for most forensic DNA probes, the autoradiogram displays two bands if the person is

heterozygous at the locus

Hornozygote a diploid organism that carries identical alleles at one or more genetic loci on its homologous chromosomes

Homozygous having the same allele at a particular locus; for most forensic DNA probes, the autoradiogram displays single bands if the person is Homozygous at the locus

Human leukocyte antigen (HLA) protein-sugar structures an the surface of most cells, except blood cells, that differ among individuals and important for acceptance or rejection of tissue grafts or organ translation; the locus of one particular class, HLA DQα, is used far forensic analysis with PCR

Locus (pl. loci) the specific physical location of a gene on a chromosome

Marker a gene with a known location on a chromosome and a clear-cut phenotype that is used as a paint of reference in the mapping of other loci

Polymerase chain reaction (PCR) an in vitro process that yields millions of copies of desired DNA through repeated cycling of a reaction that involves the enzyme DNA polymerase

Polymorphism the presence of more than one allele of a gene in a population at a frequency greater than of a newly arising mutation; operationally, a population in which the most common allele at a locus has a frequency of less than 99%

Tandem repeats multiple copies of an identical DNA sequence arranges in Direct succession in a particular region of a chromosome

Taq polymerase a DNA polymerase used to form double-stranded DNA from nucleotides and a single-stranded DNA template in the PCR technique

Variable number of tandem repeats (VNTR) repeating units of a DNA sequence for which the number varies between individuals

APPENDIX B

1. 10mg/ml Proteinnase K (stock solution)

Dissolve protenase K	10	mg
Sterile dd. H_20	1	ml
Store at -20 °C		

2. 5%Chelex

Chelex	5	g
Sterile dd. H_20	100	ml

BIOGRAPHY

Master Piyachet Stanyasuwan was bone on October 16, 1980 in Khon-Kaen Thailand. He received the Degree of Bachelor of Biology Science in 2002 from the Faculty of Science, Srinakarinwirot University, Bangkok, Thailand He enrolled at Chulalongkorn University in the graduate program for the degree of Master of Science in Medical science in 2003.