A STUDY ON THE SHORT TANDEM REPEAT SYSTEM (STRs) LOCUS DYS385 IN THAI POPULATION

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The Y-chromosomal short tandem repeat (STR) locus DYS385 can be typed using PCR amplification and separation of the resulting polymorphic fragments by non denaturing high resolution in polyacrylamide gel electrophoresis followed by silver staining. The PCR primers amplified a duplicated repeat sequence on the Y chromosome revealing a two-band pattern in male individuals. To determine the internal repeat structure as a basis for a consensus nomenclature, DNA sequence analysis was carried out after subcloning of PCR-amplified fragment revealing the uniform 4-bp repeat structure 'GAAA'. The shortest allele observed consists of 10 repeat units thus providing the basis of designation 'allele 10'. Except for isolated point mutations, no systematic differences could be observed either in the repeat sequence or in the flanking regions between the two fragments of a given individual. Thus was not possible to discriminate between the two loci of the DYS385 system.

From the population data in Thai showed that, the DYS385 is a highly polymorphic STR system with population-specific genotype distributions. Thus DyS385 is a robust investigation in forensic science.

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วรวีร์ ไวยวุฒิ : การศึกษา Short tandem repeat ตำแหน่ง DY385 ในประชากรไทย (A STUDY ON THE SHORT TANDEM REPEAT SYSTEM (STRs) LOCUS DYS385 IN THAI POPULATION.) คณะกรรมการควบคุมวิทยานิพนธ์ : วิโรจน์ ไวยวุฒิ, Dr.med., สมชาย ผลเอี่ยมเอก, M.D., สมบูรณ์ ธรรมเถกิงกิจ, M.D., 41 หน้า. ISBN 974-663-358-9

การตรวจพิสูจน์ทางนิติเวชในปัจจุบัน มีแนวโน้มใช้การตรวจพิสูจน์ทางดีเอ็นเอมากขึ้น โดยเฉพาะการตรวจ Short tandem repeat (STR) การศึกษาถึงตำแหน่ง DY385 ในประชากรไทย เป็นการศึกษาตำแหน่งเฉพาะบน Y-chromosome ซึ่งใช้การตรวจโดยวิธี Polymerase Chain Reaction (PCR) และตรวจหา allele โดยแยกด้วยกระแสไฟฟ้าบน non-denaluing polyocrylomide gel และย้อมสีด้วย silver แถบของ allele ซึ่งประกอบด้วยเบสที่เรียงตัวซ้ำกันแต่ละหน่วยเป็น GAAA จะ ปรากฏให้เห็นสองแถบในตัวอย่างดีเอ็นเอจากผู้ชายแต่ละคน

การศึกษาได้ศึกษาประชากรไทย 99 คน ที่อาศัยอยู่ในภาคกลางและไม่ได้เกี่ยวข้องเป็นญาติกัน พบว่า มีการกระจายของ allele ตั้งแต่ allele ที่ 10 จนถึง 22 โดย allele ที่ 14 จะพบมากที่สุด และ genotype 14-18% จะมากที่สุดคิดเป็น 7% ของประชากรทั้งหมด นอกจากนี้การกระจายของ allele เป็นไปตาม Hardy Weinberg Equiribrium ดังนั้น การตรวจพิสูจน์ทาง DNA ในตำแหน่ง DY385 จึงมีประโยชน์เป็นอย่างมากในการตรวจพิสูจน์ทางนิติเวช

Contents

Page

ACKNOWLEDGEMENT.....iii

ABSTRACT.....iv

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

CHAPTER

I. INTRODUCTION..... 1

1.1 Human genome and repetitive sequence..... 1

1.2 Extragenic repeated DNA sequences and transposable elements..... 2

1. 2.1 Tandem repetitive sequences..... 2

1. 2.1.2 Minisatellite DNA..... 3

1 2.1.3 Microsatellite DNA..... 4

1.3 Polymorphism of short tandem repeat..... 5

1.3.1 Slipped strand mispairing..... 5

1. 4 Application of DNA polymorphism in forensic science..... 9

1. 4.1 Identity and relationships..... 9

1. 4.1.1 Minisatellite DNA fingerprinting probe..... 9

1. 4.1.2Minisatellite Probes..... 9

1. 4.1.3 Microsatellite markers..... 10

1. 4.2 Paternity Test..... 10

1.5 The Y microsatellite locus DYS385..... 11

2. Material and Method..... 12

2.1 Material..... 12

2.1.1 Reagent and Chemical..... 12

2.1.2 DNA samples..... 12

2.2 Method..... 13

2.2.1 DNA Extraction from Fresh blood Samples..... 13

2.2.2 Polymerase Chain Reaction (PCR) 16
2.2.3 Procedure for amplification DYS385 locus by PCR 19
2.2.4 Gel electrophoresis 20
2.2.4.1 Agarose gels 20
2.2.4.2 Polyacrylamide gels 21
2.2.5 PAGE and silver staining 23
2.2.5.1 Denaturing polyacrylamide gel 23
2.2.5.2 Natived polyacrylamide gel 23
2.2.6 Silverstaining for natived polyacrylamide gel 26
2.2.6 Silverstaining for denatured polyacrylamide gel 27
3. Result 29
3.1 Electrophoresis seperation from DYS385 PCR product 29
3.1.1 Denatured Polyacrylamide gel 29
3.1.2 Native Polyacrylamide gel 29
3.2 Primer sensitivity 30
3.3 The population frequencies 31
4. Discussion 32
5. References 39
6.Biography 43

List of Figures

Fig. 1 Slippaged mispairring strand caused of addition and deletion to form the new allele.

Fig.2 Unequal crossing over

Fig.3 PCR schematic

Fig.4 Agarose's structure

Fig.5 Acrylamide and Bisacrylamide crossing-net form to polyacrylamide gel.

Fig.6 Image of DYS385 in denatured polyacrylamide gel

Fig.7 Natived polyacrylamide gelelectrophoresis for DYS385

Fig.8 Primer sensitivity test in the agarose gel

List of Table

Tab.1 The classification of repetitive tandem repeat

Tab.2 DYS385 genotype frequencies

List of Abbreviations

bp	Base pair
µl	Microlitre
DNA	Deoxyribonucleic acid
Hr	Hour
M	Molar
min	minute
ml	mililitre
mM	milimolar
°C	Dgree celcius
PCR	Polymerase Chain Reaction
Rpm	revolution per minute
Sec	second
UV	Ultra violet

CHAPTER I

INTRODUCTION

1. Human genome and repetitive sequence

Single-stranded deoxyribonucleic acid (DNA) is a long linear polynucleotide constructed from four different nucleotide monomers. The odering of these monomers defines DNA sequence. Double-stranded DNA consists two such polymers held together by the bond via specific nucleotide pairs, such that the sequence of one strand defines the sequence of its homologous strand. The resultant double stranded DNA is twisted in a helix and structurally stabilized by packing around nuclear protein call histones. The combined complex is then call chromatin. Chromatin may be condensed to varying levels of compact state as chromosomes. The DNA in the human genome is distributed unequally among 23 pairs of chromosomes, these totaling some 3.2×10^9 nucleotides per diploid genome [1]

The human genome consist of DNA sequences of known function like as genes and DNA sequences for which no clear function has been established or for which no function may exist. Such non-coding DNA may either be in single copy acting as spacer sequence between coding regions or be in multiple copies. This is termed repetitive DNA and accounts for as much as 30% of human genome with many such sequences having little or no transcriptional and translational capacity [2-8].

2. Extragenic repeated DNA sequences and transposable elements

The human nuclear genome, like that of other complex eukaryotes, contains a large amount of highly repeated DNA sequence families which are largely transcriptionally inactive. Like multigene families, they occur in two major types of organization

Tandemly repeated DNA. Such families are defined by blocks or arrays of tandemly repeated DNA sequences. Individual arrays can occur at a few or many different chromosomal locations. Depending on the average size of the arrays of repeat units, highly repetitive noncoding DNA belonging to this class can be grouped into three subclasses:

1. Satellite DNA
2. Minisatellite DNA
3. Microsatellite DNA

Interspersed repetitive DNA. The individual repeat units are not clustered, but are dispersed at numerous locations in the genome. Most of the DNA families belonging to this class contain members that are capable of undergoing retrotransposition, that is transposition through an RNA intermediate.

2.1 Tandem repetitive sequences

The human genome contains four major tandem repeat classes: Satellite I, II, III and IV [2], These total about 5% of the genome and correspond to the satellite bands separated by CsCl centrifugation of genomic DNA. Their sequences has been found to be tandem repeating sequences, whether separable by centrifugation or not. Each of the Satellites I to IV represents a heterogenous mixture of DNA species, similar in DNA base composition, and thus buoyancy, but not necessarily

similar in DNA sequence. For example, a further major tandemly repeating DNA species is the alphoid class, constituing some 2% or more of the genome [9-10]. Although mostly co-buoyant with Satellite II and III, alphoid DNA is quite distinct from these in sequence.

The consensus base sequences, that is to say the smallest tandemly repeating units of Satellites I to IV and alphoid satellite, are known[10-13] This may be as small as 5 bp in the case of Satellite III, but is more often larger, such as the 170 bp alphiod repeat, The size of this repeat units is generally contrast with in each class. However, sequence divergence with each class may generate related families of sequences [10] with different members sometimes being associated with specific chromosome.

2.1.2 Minisatellite DNA

Minisatellite DNA comprises a collection of moderately sized arrays of tandemly repeated DNA sequences which are dispersed over considerable portions of the nuclear genome (Tab. 1.). Like satellite DNA sequences, they are not normally transcribed.

Hypervariable minisatellite DNA sequences are highly polymorphic and are organized in over 1000 arrays (from 0.1 to 20 kb long) of short tandem repeat [14]. The repeat units is different hypervariable arrays vary considerably in size, but share a common core sequence, GGGCAGGAXG (where X = any nucleotide), which is similar in size and in G content to the chi sequence, a signal for generalized recombination in E.coli. While many of the arrays are found near the telomers, several hypervariable minisatellite DNA sequence occur at other chromosomal locations. Although the great majority of hypervariable minisatellite DNA sequences are not transcribed, some rare case are known to be expressed. For example, the MUC1 locus on 1q is known to encode a highly polymorphic

glycoprotein found in several epithelial tissues and body fluids as a result of extensive variation in the number of minisatellite-encoded repeats [15].

The significance of hypervariable minisatellite DNA is not clear, although it has been reported to be a hot spot for homologous recombination in human cells [16]. Nevertheless it has found many applications. Various individual loci have been characterized and used as genetic markers, although the preferential localization in subtelomeric regions has limited their use for genome-wide linkage studies. A major application has been in DNA fingerprinting, in which a single DNA probe which contains the common core sequence can hybridize simultaneously to multiple minisatellite DNA loci on all chromosomes, resulting in a complex individual-specific hybridization pattern.

2.1.3 Microsatellite DNA

Microsatellite DNA families include small arrays of tandem repeats which are simple in sequence (often 1-4 bp) and are interspersed throughout the genome.

The significance of microsatellite DNA is not known. Alternating purine-pyrimidine repeats, such as tandem repeats of the dinucleotide pair CA/TG, are capable of adopting an altered DNA conformation, Z-DNA, in vitro, but there is little evidence that they do so in the cell. Although microsatellite DNA has generally been identified in intergenic DNA or with in the introns of genes, a few examples have been recorded within the coding sequences of genes. Tandem repeats of three nucleotides in coding DNA may be sites that are prone to pathogenic expansions.

Tab. 1 The classification of repetitive tandem repeat

Class	Size of repeat unit (bp)	Major chromosomal location
Satellite DNA (blocks often from 100 kb to several Mb in length)		
Satellites 2 and 3	5	Most possibly all, chromosomes
Satellite 1	25-48	Centromeric heterochromatin of most chromosomes and other heterochromatic regions
α (alphoid) DNA	171	Centromeric heterochromatin of all chromosome
β (Sau3A family)	68	Notably the Centromeric heterochromatin of 1, 9, 13, 14, 15, 21, 22 and Y
Minisatellite DNA (blocks often within the 0.1-20 kb range)		
telomeric family	6	All telomeres
hypervariable family	9-24	All chromosomes, often near telomer
Microsatellite DNA (blocks often less than 150 bp)	1-4	All chromosomes

3. Polymorphism of short tandem repeat

3.1 Slipped strand mispairing

Germline mutation rates at a variety of microsatellite loci exhibit considerable variation, ranging from an andetectable level up to about 8×10^{3} [17,18]. Novel length alleles at (CA)/(TG) microsatellites and tetranucleotide marker loci are known to be formed without exchange of flanking markers. This means that they are not generated by unequal crossover. Instead, as new mutant alleles have been observed to differ by a single repeat unit from the originating parental allele [17] the most likely mechanism to explain length variation is a form of exchange of sequence

information which commences by slipped strand misparing. This occurs when the normal pairing between the two complementary strands of a double helix is altered by staggering of the repeats on the two strands, leading to incorrect pairing of repeats. Although slipped strand mispairing can be envisaged to occur in nonreplication DNA, replicating DNA may offer more opportunity for slippage and hence the mechanism is often also called replication slippage or polymerase slippage (Fig. 1). In addition to mispairing between tandem repeats, slippage replication has been envisaged to generate large deletions and duplications by mispairing between noncontiguous repeats and has been suggested to be a major mechanism for DNA sequence and genome evolution [19,20].

Unequal crossover is a form of recombination in which the crossover takes place between nonallelic sequences on nonsister chromatids of a pair of homologs. Often the sequences at which crossover takes place show very considerable sequence homology which presumably stabilizes mispairing of the chromosome. Because crossover occurs between mispaired nonsister chromatids the exchange results in a deletion on one of the participating chromatids and an insertion on the other. The analogous exchange between sister chromatids is called unequal sister chromatid exchange [21] (Fig. 2.). Both mechanisms occur predominantly at locations where the tandemly repeated units are moderate to large in size. In such cases the very high degree of sequence homology between the difference repeats can facilitate pairing of nonallelic repeats on nonsister chromatids or sister chromatids. If chromosome breakage and rejoining occurs while the chromatid are mispaired in this way, an insertion or deletion of an intergral number of repeat units will result. Unequal sister chromatid exchange is thought to be a major mechanism underlying VNTR polymorphism in the rDNA clusters. Unequal crossing over is also expected to occur comparatively frequently in complex satellite DNA repeats and at tandemly repeated gene loci.

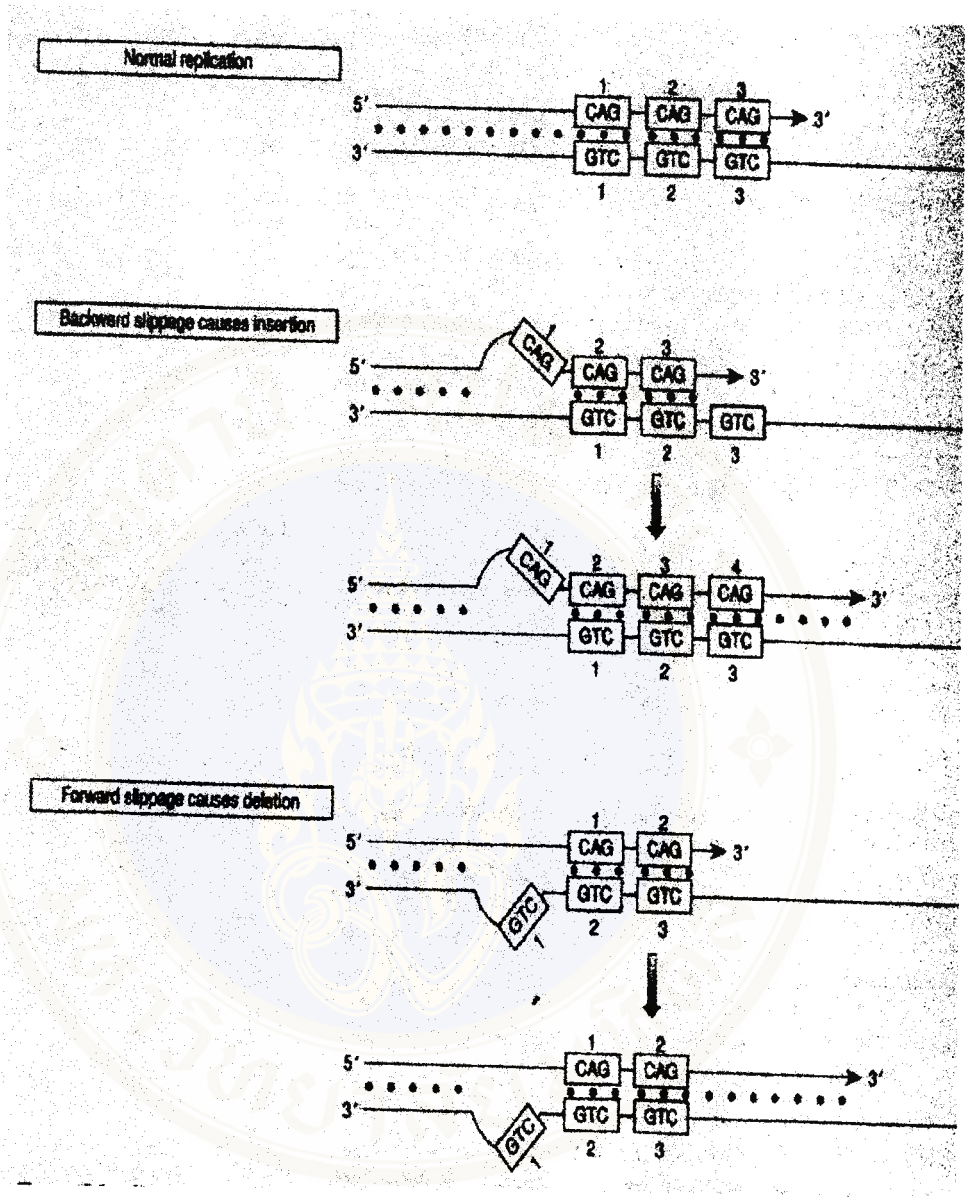


Fig. 1 Slippaged mispairring strand caused of addition and deletion to form the new allele.

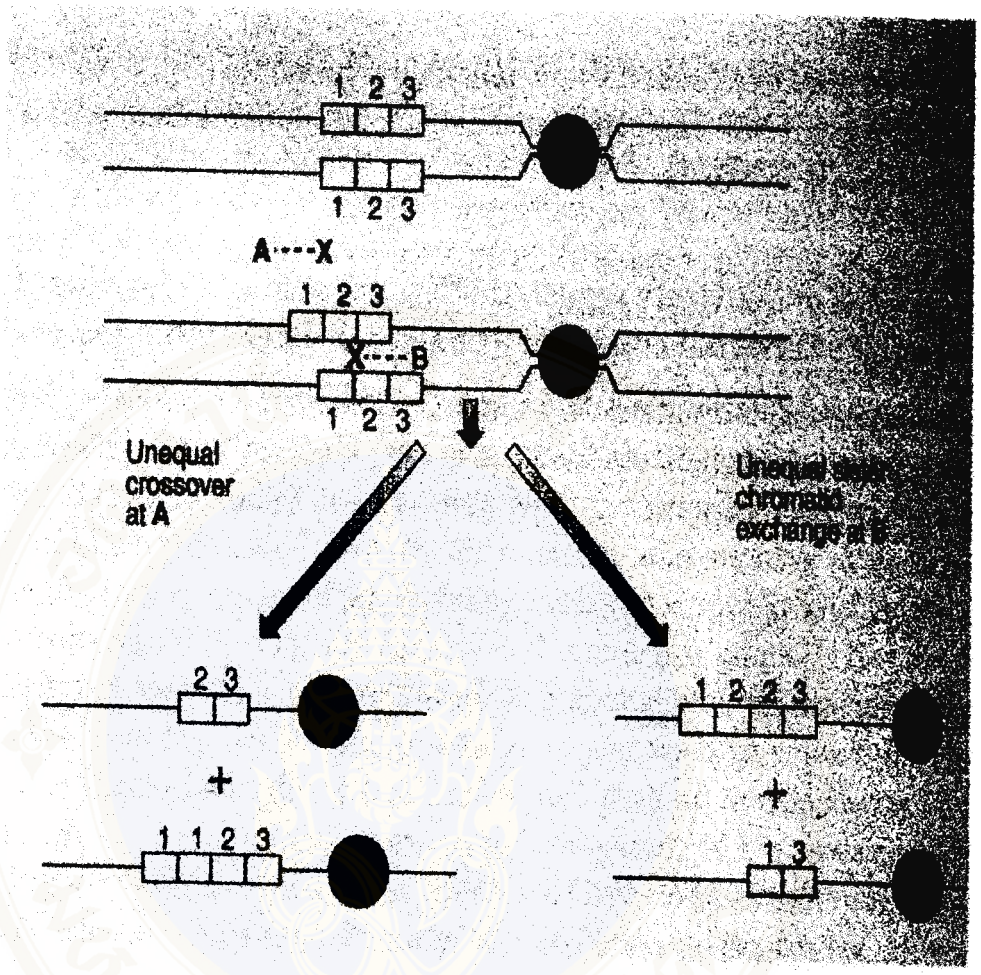


Fig. 2 Unequal crossing over

4. Application of DNA polymorphism in forensic science

4.1 Identity and relationships

The term of DNA profiling was used to refer to the general use of DNA tests to establish identity or relationships Three types of genetic markers are widely use, as described in term:

Minisatellite DNA fingerprinting probe

Minisatellite probe

Microsatellite markers

4.1.1 Minisatellite DNA fingerprinting probe

These probes contain the common core sequence of a hypervariable dispersed repetitive sequence (GGGCAGGANG), first discovered by Jeffreys et al, 1985 in the myoglobin gene. They give and individual-specific fingerprint of bands when hybridized to Southern blots. Their chief disadvantage is that it is not possible to tell which pairs of bands in a fingerprint represent alleles. Thus, when matching DNA fingerprints, one compares the position and intensity of each band individually. Other hypervariable repeated sequences have been used in the same way, for example those detected by the synthetic oligonucleotide $(CAC)_5$.

4.1.2Minisatellite Probes

Minisatellite or VNTR probes recognize single-locus hypervariable tandem repeats on Southern blots. Each person's DNA should give two bands, representing the two alleles. Profiling is based on four to 10 different VNTR polymorphisms. These probes allow exact calculations of probabilities if the gene frequency of each allele in the population is known. For matching alleles between different gel tracks, the continuously variable distance along the gel has to be divided into a number of bins. Bands falling within the same bin are deemed to match. It is imperative that the criteria use for judging matches in each profiling test should be the

same binning criteria that were used to calculate the population frequencies of each allele. The binning criteria can be arbitary within certain limits, but they must be consistent.

4.1.3 Microsatellite markers

Microsatellite polymorphisms are based on short tandem repeats, usually di-, tri- or tetranucleotides. They have the advantages over minisatellites that they can be typed by PCR and that discrete alleles can be defined unambiguously by the precise repeat number. This avoids the binning problem that makes it easier to relate the results to population gene frequencies. Minor variations within repeated units of some microsatellites potentially allow and almost infinite variety of alleles to be discriminated, so that the genotype at a single locus might suffice to identify an individual [22].

4.2 Paternity Test

Exclude paternity is fairly simple – if the child has a marker allele not present in either the mother or alleged father then, barring new mutation, the alleged father is not the biological father. Proving paternity is in principle of Medelian theory, that rules everyone has two copies of their genetic information that half from each parents. Now the microsatellite markers have been widely used for this purpose.

5. The Y microsatellite locus DYS385

DNA typing using short tandem repeat (STR) loci has become the method of choice in forensic stain analysis. Autosomal STR loci exhibit a Mendelian type of inheritance with two codominant alleles present at each locus, and are subject to normal recombination and mutation events. In contrast, Y-chromosomal short tandem repeats are present only in male individuals with single alleles [23], as they are located on the non-recombining part of the Y-chromosome. Thus the Y-STRs are segregating as closely linked haplotypes in the male lineage of a family [23-25].

Whereas most Y-linked STR systems only exhibit a single polymorphic fragment which can be amplified by PCR, a number of Y-STRs originate from a duplicated tandem repeat array, as two fragments of variable size are obtained by using a single primer pair e.g. DYS389 I / II and YCA I / II [26,27], thus giving the impression of a typical autosomal two-allele genotype. However, these two fragments are linked and have to be regarded as a single "allele" or "haplotype" composed from two fragments. The STR locus DYS385 also belongs into this category, as it is characterized by a highly variable duplicated repeat sequence on the human Y-chromosome revealing a two-band pattern in male individuals [25]

CHAPTER II

MATERIAL AND METHODS

1 Material

1.1 Reagent and Chemical

Enzyme	
Taq Polymerase (5U/μl)	Gibco
Primer	
(DYS385-1)	
5'- AGC ATG GGT GAC AGA GCT A-3'	Gibco
(DYS385-2)	
5'- GGG ATG CTA GGT AAA GCT G-3'	Gibco
Other reagent	
Rotiphorese Gel 40 (Acrylamide:Bis 19:1)	Roth
TEMED	Pharmacia
Agarose	Biozym
Chelex®100	Biorad

1.2 DNA samples

For population study the randomly selected unrelated male individual were studied. 99 male blood donors from Bangkok, Thailand were chosen to prepare for genomic DNA and analysed for DYS385 locus.

2 Method

2.1 DNA Extraction from Fresh blood Samples

Principle

The isolation of genomic deoxyribonucleic acid (DNA) is a crucial step in the process of DNA profiling. The success of all subsequent genetic-typing procedures depends on the availability of sufficient amounts of highly purified DNA from blood samples.

Blood is composed from white and red blood cells. The erythrocyte was lysed by hypotonic solution and next to the white blood cells. The DNA is free from Protein by phenol-chloroform extraction or NaCl-precipitation [28].

Method

Purification of White Blood Cells

1. Place 10-15 ml EDTA blood into 50 ml polypropylene tube.
2. Add 50 mM KCl up to 50 ml, mix well, and incubate in water bath at 37°C for 10 min.
3. Spin for 10 min at 500 g in a clinical centrifuge at room temperature
4. Remove the supernatant with the Pasture pipet connected to a water jet or vacuum pump; leave pellet intact (hold the tube in front of a light source to see the white-cell pellet).
5. Repeat step 2-4 once or twice until the pellet is free from red cells.
6. Add 15 ml lysis buffer and shake vigorously to resuspend the cell pellet.
7. Add 1.5 ml 10% SDS and mix carefully. The sample should now become very viscous because of cell lysis
8. Incubate overnight at 37°C or at 55°C for 4-5 hours

Organic Extraction of DNA

1. Add 1 vol. Buffered phenol to the protinase K-digested cell extract and mix the aqueous and organic phases carefully to achieve a homogenous suspension; avoid vigorous shaking.
2. Spin for 10 min at 1500 g in clinical centrifuge at room temperature.
3. The aqueous phase containing the DNA is on top, and the phenolic phase to a fresh 50-ml polypropylene tube using a wide bore glass pipet, The interphase containing proteins and protein-DNA complexes may also be transferred at this step.
4. Add 1 vol of 1:1 mixture of phenol and chloroform/ isoamyl alcohol (24:1) and extracts as described in step 1-3.
5. Add 1 vol of chloroform/ isoamylalcohol(24:1)and repeat the extraction as described in steps 1-3. At this step, avoid transferring any residual protein debris from the interphase.

Inorganic Extraction of DNA

1. Add 5 ml of 6 M NaCl (one-third of the origin volume) to the proteinase K-digested cell extract and shake vigorously for 10-15 sec.
2. Spin for 10 min at 1500 g in a clinical centrifuge at room temperature to separate the salt-precipitated proteins.
3. Pour the supernatant into a fresh 50 ml tube.

DNA Precipitation

1. After organic extraction, add 0.1 vol of 3 M sodium acetate and 2 vol of iced-cold absolute ethanol. After salt precipitate with 6 M NaCl, add only the ethanol.
2. Mix carefully without vigorous shaking. The DNA should precipitate by forming viscous strings first and finally a compact pellet, which may float on top of the solution.
3. Melt the tip of Pasture pipet on a Bunsen burner to form a hook. Use this tool to recover the floating DNA pellet from the solution.

4. Rinse the DNA pellet attached to the glass hook twice in 70% ethanol to remove excess salt.
5. Dry the pellet brifely in the air and resuspend the DNA in an appropriate volume (300-500 µl depend on the size) of 0.1X TBE.
6. Incubate the sample for 1 hour at 65°C until a homogenous solution is obtained.

Chelex Extraction of Whole Blood

Principle

The chelating-resin-based procedure is a simple, one-tube minimal step extraction process that requires very little time. The risk of operator-induced error, such as contamination or sample mix up, is also reduced, since the procedure requires fewer manipulations. Most importantly, the use of a chelating resin to extract DNA eliminates the use of hazardous chemicals, such as phenol-chloroform.

The simplicity of chelating-resin-extraction methods has made this extraction method very desirable in the forensic community. DNA has successfully been extracted using Chelex® 100 (Bio-rad Laboratories, Hercules, CA), a chelating resin, from a variety of forensic samples. Chelex 100 scavenges metal contaminants to an extremely high degree of purity without altering of the concentration of nonmetalic ions [29]. The resin is composed of styrene divinylbenzene copolymers containing paired iminodiacetate ions. Chelex 100 has a particularly high selective for divalent ions and differs from ordinary ionexchangers because of its high selectivity for metal ions and its higher bond strength[30].

Method

1. Pipet 1 ml of sterile deionized water into a 1.7 ml-microcentrifuge tube.
2. Add approximate 3 µl of whole blood, mix and incubate at room temperature for 15-30 min.
3. Prepare a 5% Chelex solution in sterile deionized H_2O.
4. Centrifuge samples for 3 min at 10,000g in a microcentrifuge to pellet the red blood cells.
5. Carefully remove all but approximate 20-30 µl of the supernatant and discard. Leave the substrate and pelleted material in the tube.
6. Resuspend the pellet in 5%Chelex 100 to final volume of 200 µl
7. Incubate at 56°C for 30 min.
8. Vortex at high speed 5 sec.
9. Centrifuge samples for 3 min at 10,000g to pellet the Chelex 100 resin and substrate. The extracts are now ready for quantitation and the PCR amplification process.

2.2 Polymerase Chain Reaction (PCR)

PCR is a rapid and verstile in vitro method for amplifying defined target DNA sequences present within a source of DNA [31]. The method is designed to permit selective amplification of a specific target DNA sequence or sequences within a heterogenous collection of DNA seqences. To permit the selective amplification, some prior DNA sequence informatin from the target sequences is required, enabling the construction of two oligonucleotide primer sequences. Such so called amplimers, when added to denatured genomic DNA, will bind specifically to complementary DNA sequences immediately flanking the desired target region. They are designed so that, in the presence of a suitably heat-stable DNA polymerase and DNA precursors (the four deoxynucleoside triphosphates, dATP, dCTP, dGTP and dTTP), they can initiate the synthesis of new DNA

strands which are complementary to the individual DNA strands of the target DNA segment, and which will overlap each other.

PCR is a chain reaction because newly synthesized DNA strands will act as templates for further DNA synthesis in subsequent cycles. After about 30 cycles of DNA synthesis, the products of the PCR will include, in addition to the starting DNA, about 10^5 copies of the specific target sequence, an amount which is easily visualized as a discrete band of a specific size when submitted to agarose gel electrophoresis. A heat-stable DNA polymerase is used because the reaction involves sequential cycles composed of the three step:

1. Denaturation: The separation of double strand to single strand.
2. Anealing The binding of Primer to each strand.
3. Extension The elongation of new strand complement to template.

The temperature and length of time from each step is different. In denaturation cycle temperature was up to 93-95°C for break down the hydrogen bond. In the anealing cycle temperature was depend on the melting temperature (Tm) from each primer followed through number of A, C, G and T in primer sequence, that can calculated in form: Tm = [(number of A+T) × 2°C] + [(number of G+C) × 4°C].

In step of extension cycle the temperature laying on 72°C.

The copy numbers or PCR products can calculated by the formular

$$X = (2^n - 2n)$$

n = number of cycles

2n= nonspecific products

X = specific products

In this study the Touchdown-program [32] was performed to increase the specific products.

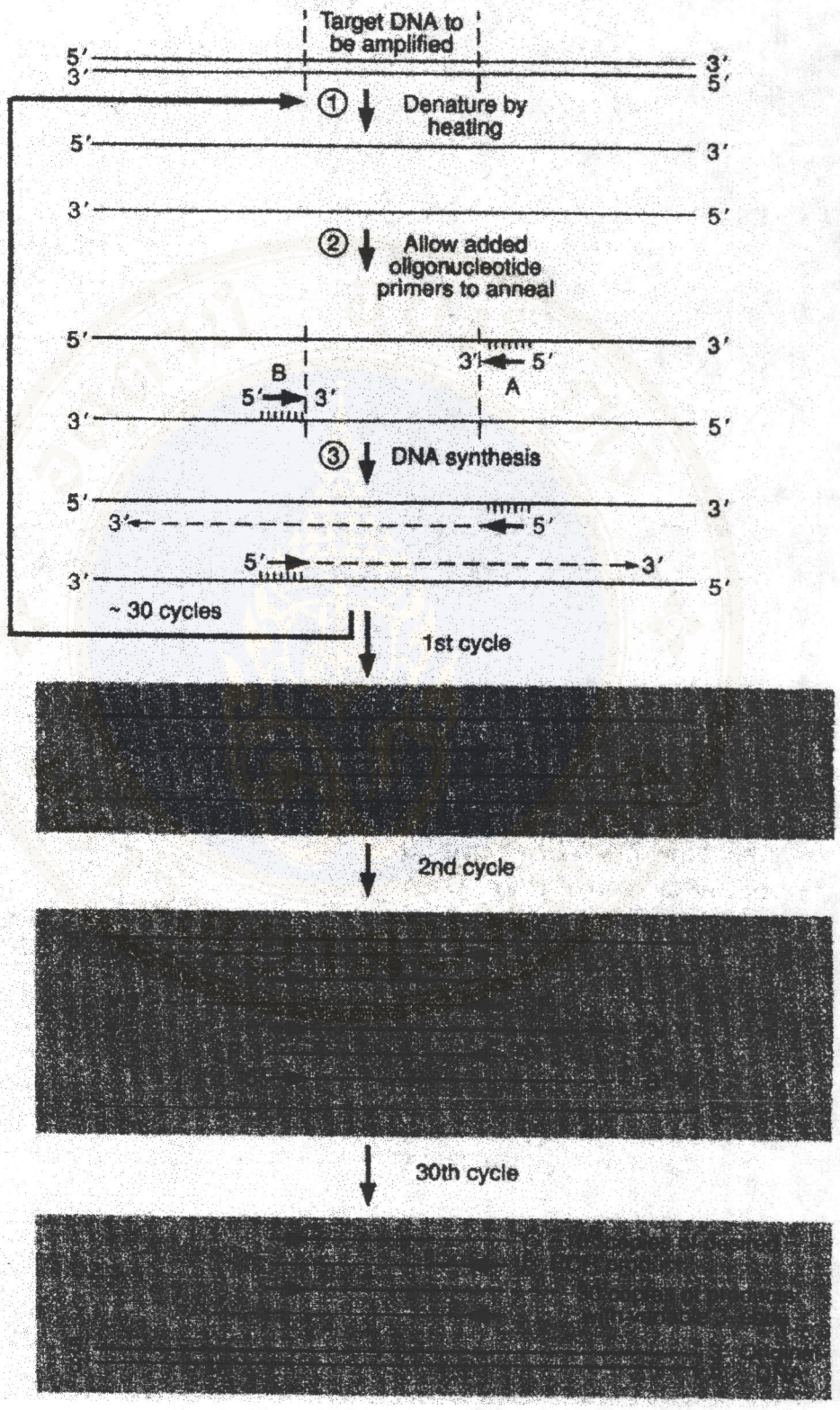


Fig. 3 PCR schematic

2.3 Procedure for amplification DYS385 locus by PCR

The PCR was performed in 100 µl reaction mix containing

Template DNA (10ng/µl)	10µl
10 × PCR buffer	10µl
dNTPs (dATP, dCTP, dGTP, dTTP) each 200µm	8µl
$MgCl_2$ (50mM)	3µl
Primer (DYS385 1 and 2) each 25 mM	each 1µl
Taq polymerase (5U/µl)	0.5 µl
H_2O	66.5 µl

The PCR amplification protocol was carried out as follows (using a 2400 thermocycler, Perkin Elmer) initial denaturation for 3 min at 94°C, then a touchdown PCR with denaturation for 30 sec at 94°C, anealing for 30 sec at decreasing temperatures of 59-57°C (3×2 cycles at each temperature), extension for 1 min at 72 °C, followed by 29 cycles as above with 56°C anealing temperature, and a final extension for 7 min at 72°C.

Primer for DYS385 locus

(DYS385-1) —5'- AGC ATG GGT GAC AGA GCT A-3';

(DYS385-2) –5'- GGG ATG CTA GGT AAA GCT G-3'

2.4 Gel electrophoresis

2.4.1 Agarose gels

Agarose gels are mostly used when large pores for the analysis of molecules over 10 nm in diameter are needed. Agarose is a polysaccharide obtained from red seaweed.

By removal of the agaropectin, gels of varying electroosmosis and degrees of purity can be obtain. They are characterized by their melting point (35°C to 95°C) and the degree of electroendosmosis.

The pore size depends on the concentration of agarose. Agarose is dissolved in boiling water and then forms a gel upon cooling. During this process double helixes which are joined laterally to form relatively thick filaments (Fig. 4)

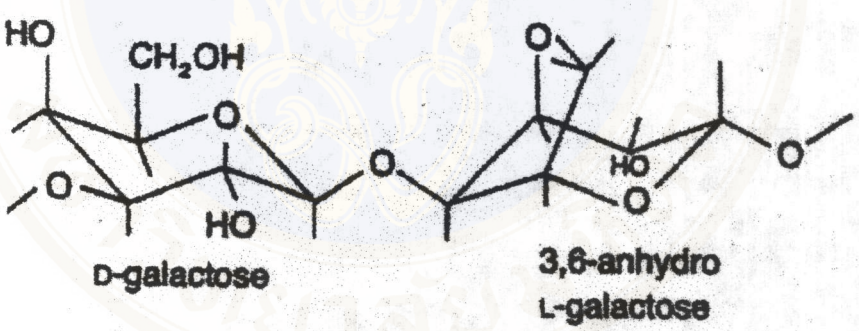


Fig.4 Agarose's structure

For DNA seperations 1 to 10 mm thick gels are cast on UV-transparent trays, because the bands are usually stained with fluorescent dyes (Ethidium bromide).

In this study 1% Aagarose gel with Ethidiumbromide-TBE-buffer (40µl Ethidium-bromide/l 1×TBE was performed and prepared in minigel (8×10 cm^2) to analyze quantity and quality of PCR product. 12 µl of PCR product was loaded with 2 µl 5×loading buffer in the minigel. By 1×TBE in 50 Volt about 30 min. the PCR product can seen under UV light (312 nm in wavelength).

5×Loadingbuffer:

0.25%	Bromphenol blue
0.25%	Xelenecyanol
30%	Glycerine

5×TBE

51g	Tris
27.5g	Boric acid
3.75g	EDTA

add H_2O to 1 L and tritrate with HCL to pH 8

2.4.2 Polyacrylamide gels

Polyacrylamide gel was first used for electrophoresis in 1959, which are chemically inert and particularly mechanically stable. By chemical co-polymerization of acrylamide monomers with a cross-linking reagent usually N,N'-methylene-bisacrylamide (Fig. 5) a clear transparent gel with exhibits very little electroendosmosis is obtained.

The pore size can be exatly and reproducibly controlled by the total acrylamide concentration T and the degree of cross-linking C

Electrophoretic separation of amplified PCR products was perform using a vertical SA-32 apparatus (Life Technologies GmbH), which the size is 32 × 19 cm and 0.8 mm in thickness.

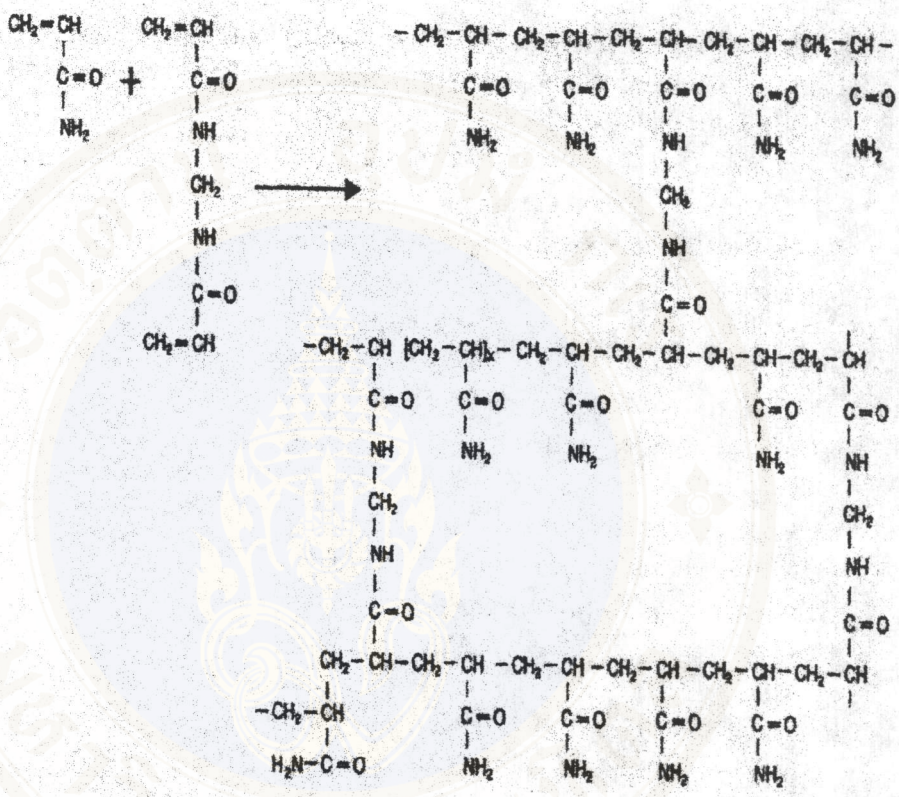


Fig. 5 Acrylamide and Bisacrylamide crossing-net form to polyacrylamide gel.

2.5 PAGE and silver staining

Many new techniques and applications have recently been developed in this field. Because those are almost exclusively based on PCR technology, the size range of the DNA fragments to be analysed lies between 50 and 1500 bp. In this range the sensitivity and resolution of agarose electrophoresis with Ethidiumbromide staining is coming to its limit, because the gel pores are too large for proper sieving and the intercalating fluorescent dyes are much less sensitive than for lager fragments.

The use polyacrylamide gels lead to much sharper bands and higher resolution; with subsequent silver staining a sensitivity of 15 pg per band can be achieved. Vertical and horizontal slab gels can be used. Where as in agarose electrophoresis the mobilities of DNA fragments are solely proportion to their sizes, the band positions in polyacrylamide gels are partly influenced by the base sequence as well. A and T rich migrate slower than others.

Silver stained DNA band can directly reamplified after scratching them out of gel without intermediate purification. About 20% of the DNA molecules of a band remain undestroyed by the silver staining procedure. They are locked inside the stained band, thus DNA fragments do not contaminate the gel surface during staining.

2.5.1 Denaturing polyacrylamide gel

Denaturing gels are used to size-fractionate single stranded DNA or RNA molecules. The initial nucleic acid sample may be double-stranded and requires to be denatured prior to loading on the gel. The gel also contains a high concentration of chemical denaturant to ensure that any complementary strands remain single-stranded. The chemical denaturants used are strongly polar simple molecules, typically containing amino

(NH2) and carbonyl (C=O) groups. Such chemical group effectively compete for hydrogen bond formation with the amino and carbonyl group of bases, and so can disrupt the hydrogen bonding between base pairs. Frequently used denaturants in gel include urea, formamide and formaldehyde.

In this study 4% polyacrylamide gel with 8M urea was performed to use in separation the PCR product from DYS385. The PCR products were loaded between 1.5 to 4 µl by concentration, that measured from 1% agarose gel, and mixed by loading buffer 1:1. The mixture was denatured by 95° for 5 min before loading on the gel and run by 1000 volt about 90 min.

4% polyacrylamide solution

31.5g	Urea
7.5 ml	5×TBE buffer
7.5 ml	40% (w/v) Acrylamide: Bisacrylamide 19:1
36.5 ml	H_2O
500 µl	10% Amoniumpersulphate
50 µl	TEMED (N,N,N',N'-trtramethylethylenediamine)

5×TBE buffer

54g	Tris
27.5g	Boric acid
3.75g	EDTA

add H_2O to 1 litre and tritrate with HCl to ph 8

Loading buffer for Denatured polyacrylamide

0.1%	Bromphenol blue
0.1%	Xylenecyanol

in formamide

2.5.2 Natived polyacrylamide gel

Electrophoretic separation of PCR product was test together by 4% native polyacrylamide gel. As like as the denature polyacrylamide gel the PCR products were loaded between 1.5 to 4 µl and mixed by 1 µl loading buffer. To avoid the unsharp band 200 volt was applied to run about 15 hours in 1×TBE.

4% polyacrylamide solution

12 ml	5×TBE buffer
7.5 ml	40% (w/v) Acrylamide: Bisacrylamide 19:1
40.4 ml	H_2O
500 µl 1	10% Amoniumpersulphate
50 µl	TEMED (N,N,N',N'-trtramethylethylenediamine)

1×TBE buffer

10.8 g	Tris
5.5g	Boric acid
0.75g	EDTA

add H_2O to 1 litre and tritrate with HCl to ph 8

Loading buffer for Natived polyacrylamide gel

0.25% Bromphenol blue

0.25% Xylenecyanol

30% Glycerine

2.6 Silverstaining for natived polyacrylamide gel

The silver stained for 4% natived polyacrylamide gel was performed form fixing, rinse, staining, washing, developing and stop reaction step by step as in a table

Step	**Solution**	**Time (min)**
Fixing	Solution A	25
Rinse	H_2O	5
Staining	Silvernitrate	30
Washing	H_2O	< 10 sec.
Developing	Solution B	10-15
Stop	Solution C	60

Solution A

25% Methanol

1% HNO_3

Silvernitrate

0.1% $AgNO_3$

Solution B

3% Na_2CO_3

0.017% Formaldehyde (CH_2O)

Solution C

2% Acetic acid

2% Glycerine

2.6 Silverstaining for denatured polyacrylamide gel

The staining for denatuerd polyacrylamide gel was modified from Sammons et al, 1981 [33]. The six step performed as in a table.

Step	**Solution**	**Time (min)**
Fixing	Solution A	25
Rinse	H_2O	5
Staining	Silvernitrate	20
Washing	H_2O	< 10 sec.
Developing	Solution B	< 5
Stop	Solution C	60

Solution A

10% Ethanol

0.5% HNO_3

Silvernitrate

0.1% $AgNO_3$

Solution B

1.5% Na_2CO_3

0.01% $NaBH_4$

0.4% Formaldehyde (CH_2O)

Solution C

2% Acetic acid

2% Glycerine

CHAPTER III

RESULT

1 Electrophoresis seperation from DYS385 PCR product

1.1 Denatured Polyacrylamide gel

From the denatured polyacrylamide gel DNA migrated through it in single strand form. Every band in gel from each allele showed split band like two alleles and too difficult to allele typing (Fig. 6).

Fig.6 image of DYS385 in denatured polyacrylamide gel showed two bands in each allele

1.2 Native Polyacrylamide gel

In the native gel (without urea) DNA fragment was migrated in double-strand form. The concentration of 4% polyacrylamide was prepared to separate PCR fragment. After stained by silver the PCR –fragmented showed good image for allele typing (Fig. 7). Then 4% native gel was used in this study to typing the allele in Thai population.

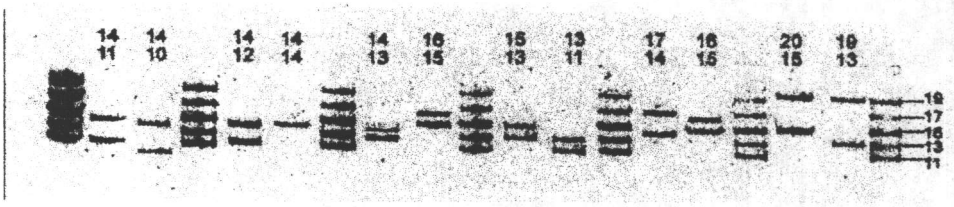


Fig. 7 Natived polyacrylamide gel electrophoresis for DYS385

2 Primer sensitivity

The concentration of DNA were used in different from 1ng to 100 μg to amplified with PCR method. PCR products were loaded in 1% Agarose gel to optimize quantity of product. The lowest sensitivity from template DNA for successful amplified was limit at 5ng (Fig. 8). In the lane of 100 and 50 pg enabled to be identified band of PCR product.

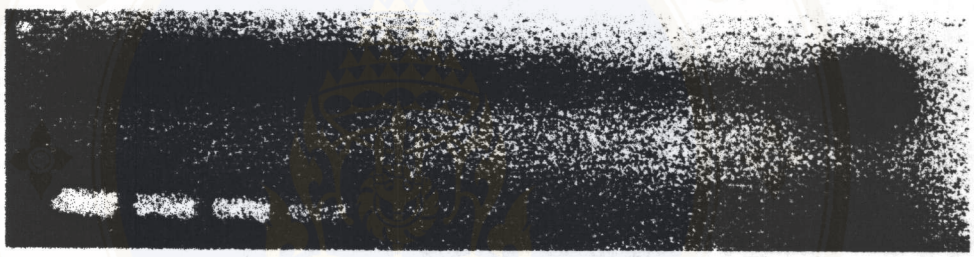
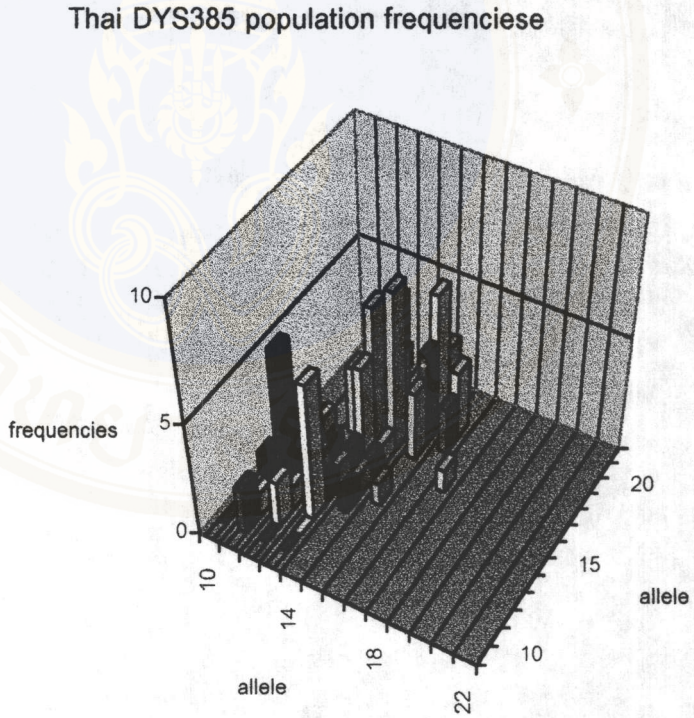


Fig. 8 Primer sensitivity test in the agarose gel

3 The population frequencies

The results of the population studies of Thai male 99 individuals are summarized in Table. 2 Overall 44 genotypes of 120 probably genotypes (allele 9-23) were found in population. The allele 10-22 were defined with the allele 13 were most frequent present in 37% and the other allele were present in the range of 25% in allele 14, 23% in allele 15 to 2% of allele 10. The genotypes were carrying out of frequencies 0.07 in genotype 14-20 and 0.06 in 11-14, 13-13, 13-18 and 15-20 and the rest in range of 0.01 – 0.04.



CHAPTER IV

DISCUSSION

DNA sequence from DYS385 short tandem repeat was carried out from 14 different allele representing the allelic repeats 9–23, according to a published nomenclature used previously [34]. The results were compared to the two sequences deposited in the Genome Database comprising the regions upstream (L36701) and downstream (L36702) to the tandem repetitive region. The tandem repetitive region could be subdivided into two different repeat types of 4 bp length: whereas the first repeat "AAGG" was not polymorphic in all samples studied and was always present six times, the second repeat "GAAA" is variable forming to basis of the DYS385 polymorphism. The shortest allele observed consisted of ten repeat units thus providing the basis for the designation "allele 9" with a length of 360 bp according to the nomenclature recommendations of the International Society for Forensic Haemogenetics for STR systems [35]. The longest fragment contained 22 repeat units with a total length of 412 bp (including flanking region). The complete sequence of allele 9 has been deposited in the EMBL DNA sequence database (accession no.Z93950).

No variations were observed in the upstream or downstream flanking regions of the central repeat arrays either between the same alleles of unrelated persons or between the two different alleles of the same person. Thus it was impossible to discriminate between the fragments of the two tandem duplicated loci giving rise to the typical DYS385 two-band pattern. Since fragments of identical or different length might originate from these two loci, and cannot be assigned to one or the other locus based on sequence differences, the DYS385 genotypes cannot be interpreted as "haplotypes" for linkage or phylogenetic studies. Therefore, in terms of linkage the genotype DYS385*11-14 could originate from the combination of allele 11 at the hypothetical DYS385 locus I and allele 14 at locus II, or

vice vera. These two alternative combinations would represent in fact two different haplotypes.

Using the cloned allelic fragments 11, 13, 15, 17, and 19, an allelic ladder was constructed by separate reamplification of the inserts and subsequent mixing of equal amounts of the respective ladder fragments. Due to the large size of the DYS385 fragments, a ladder representing all alleles could not separated satisfactorily in the non-denaturing PAA gel. The separation of the DYS385 fragments in a denaturing gel system produced a four-band pattern with overlapping fragment positions after silver staining due to the strong differences in A-T content between the two strands of the same DNA fragments (not shown).

The results of Thai population studies were compared with German, Chinese and Japanese [35] male individuals and summarized in Table . As the two fragments of each genotype originate from two closely linked loci as discussed above, individual allele frequencies cannot be calculated. In addition to alleles 10 to 22 defined in the sequencing study, the long alleles 23 and 24 were detected in the Chinese population. Alleles 21 to 24 were observed only in one of both of the Asian populations. Overall, 69 different genotypes were found. Of these, 36 were observed in Germans, 36 in Chinese, 33 in Japanese and 44 in Thai. In Germans, genotypes carrying the allele 14 were most frequent with 62.7% of all individuals studied. In Chinese, allele 13 was present in 36% and in Thai in 37% of the individuals studied. Allele 10 was observed in 37% of Japanese. As can be seen in Table , a number of genotypes was exclusively observed in only one of these four populations: the genotypes 11-13, 15-16/17, 16-17/18 and 17-18/19 were found only in Germans, 16-16, 11-18/19/20/21 and 12-17 only in Chinese, 10-17/18/19/20/21 and 16-21 only in Japanese, and 10-16, 11-17, 12-21, 14-21, 15-18/19, 15-21/22 and 16-19 only in Thai. Interestingly, the genotypes with allele 10 observed exclusively in Japanese were quite common in this population accounting for 34% of the

individuals studied thus providing evidence for the large genetic distance to the Asian mainland. As the majority of genotypes studied in the Asian populations has frequencies of 2% of less, it can be assumed that a number of allelic combinations has not yet been detected due to the relatively small population sample sizes in the present study.

Whereas in Germans only one common genotype was present (11-14; 33.8% frequency), the frequencies were more evenly distributed among Chinese with the 13-13 (9%), Japanese with the 13-17 (14%) and Thai with the 14-18 genotypes (7%) being most common. This is reflected by the differences regarding the mean exclusion chances, which were calculated for each population based on gene diversity values according to Nei [36]. Whereas DYS385 has an exclusion chance of 0.87 in Germans, these values are 0.95 in Chinese, 0.93 in Japanese and 0.96 in Thai. Genotype frequencies were strikingly different between the four populations studied. Therefore, evolutionary studies may be carried out based on frequency differences of Y-linked polymorphisms [37], as discussed for the Y-chromosomal STR systems [38] or diallelic markers as the Y-linked Alu polymorphic (YAP) insertion DYS287 [39,40].

The extensive genetic heterogeneity of the DYS385 system is confirmed by the recently published Y-chromosomal STR multicenter study [25,38] , which also provides population data for DYS385 already based on the nomenclature described here.

The all data showed the robust useful DYS385 to apply in forensic investigation like as person identification, parternity test etc.

Tab. 2 DYS385 genotype frequencies

DY385 Genotype	Thai (n=99)	Japaneses (n=100)	Chinese (n=100)	German (n=519)
9-17				0.0019
10-10				0.0039
10-12				0.0039
10-13				0.0096
10-14	0.01			0.0043
10-15		0.02		
10-16	0.01	0.01		
10-17		0.04		
10-18		0.07		
10-19		0.10		
10-20		0.12		
10-21		0.01		
11-11	0.02	0.01	0.04	0.0096
11-12	0.01		0.03	0.0077
11-13				0.0366
11-14	0.06		0.01	0.3353
11-15	0.01			0.0520
11-16	0.01	0.01	0.02	0.0096
11-17	0.01	0.02		0.0019
11-18		0.01	0.02	
11-19		0.03	0.01	
11-20			0.01	
11-21			0.01	
12-12	0.02		0.07	0.0077
12-13	0.01	0.01	0.02	0.0212
12-14	0.02			0.0424
12-15	0.01		0.01	0.0135

DY385 genotype	Thai (n=95)	Japanese (n=100)	Chinese (n=100)	German (n=519)
12-16	0.01	0.05	0.06	0.0173
12-17		0.08	0.01	0.0019
12-18	0.03	0.02	0.04	
12-19	0.01	0.01	0.04	0.0019
12-20	0.04		0.02	0.0019
12-21	0.01			
12-22	0.01		0.01	
13-13	0.06	0.02	0.09	0.0116
13-14	0.01	0.01	0.01	0.0559
13-15	0.01	0.03	0.01	0.0328
13-16	0.01	0.01	0.05	0.0096
13-17	0.04	0.14	0.07	0.0116
13-18	0.06	0.03	0.05	0.0116
13-19	0.03	0.01	0.08	0.0039
13-20	0.05		0.06	
13-21	0.03		0.02	
13-22			0.01	
13-23			0.01	
13-24			0.01	
14-14	0.01	0.01	0.01	0.0308
14-15	0.01	0.01		0.0366
14-16	0.01	0.01		0.0154
14-17	0.01	0.01		0.0250
14-18	0.07	0.07	0.02	0.0039
14-19	0.01	0.01	0.01	0.0019
14-20			0.01	
14-21	0.01	0.01		
14-22	0.01	0.01	0.01	

DY385 genotype	Thai (n=95)	Japanese (n=100)	Chinese (n=100)	German (n=519)
15-15	0.01	0.01		0.0231
15-16				0.0077
15-17				0.0116
15-18	0.03	0.03		0.0058
15-19	0.04	0.04		0.0019
15-20	0.06	0.06		0.0019
15-21	0.03	0.03		
15-22	0.01	0.01		
16-16			0.03	0.0039
16-17				0.0077
16-18				0.0135
16-19	0.02	0.02		0.0058
16-20	0.03	0.03		0.0019
16-21				
16-22				0.0019
17-17	0.01	0.01		0.0019
17-18				0.0212
17-19				0.0077
18-18				0.0019
18-19	0.01		0.01	0.0058

REFERENCES

1. Olmo, E., Nucleotide and cellsize in vertebrates: a review. Basic App. Histochem 1983, 27: 227-256
2. Singer, M. F. Highly repeated sequences in mamalian genomes. Int. Rev. Cytol. 1982,76: 67-112
3. Hardman, N. Structure and function of repetitive DNA in eukaryotes. Biochem. J 1986,. 234: 1-11
4. Jelinek, W. R., Schmid, C. W. Repetitive sequences in eukaryotic DNA and their expression. Ann. Rev. Biochem. 1982, 51: 813-844
5. John, B., Miklos, G. L. G. Funtional aspects of satellite DNA and heterochromatin. Int. Rev. Cytol. 1979, 58-114
6. Beridze, T. (1986) Satellite DNA. Springer –Verlag, Berlin
7. Deininger, P. L., Daniel, G. R. The recent evolution of mamalian repetitive DNA elements. Trends Genet. 1986, 2: 76-80
8. Verma, R. S. Heterochromatin of the human genome. ISI Atlas Sci. Biochem. 1988, 1: 134-138
9. Alexandrov, I. A., Mitkevich, S. P., Yurov, Y. B., The phylogeny of human chromosomespecific alpha satellites. Chromosoma 1988, 96: 443-453
10. Willard, H. F., Waye, J. S. Chromosome-specificsubsets of human alpha satellite DNA: Analysis of sequence divergence with in and between chromosomal subsets and evidence for an ancestral pentameric repeat. J. Mol. Bio. 25:207-214
11. Prosser, J., Frommer, M. Paul, C. Vincent, P. C. Sequence relationships of three Human satellite DNA's. J. Mol. Bio. 1986, 187: 145-155
12. Frommer, M., Prosser, J., Tkachuk, D., Reisner, A. H., Vincent, P. C. Simple repeated sequences in human satellite DNA. Nucl. Acids. Res. 1982, 10: 547-563

13. Holis , M., Hindley, J. Satellite II of human lymphocytes: tandem repeats of a simple sequence elements. Nucl. Acids. Res. 1998, 16:363

14. Jeffreys, A. J. Highly variable minisatellites and DNA fingerprints. Biochem Soc. Trans. 1987, 15: 309-316

15. Stroker, N. G., Cheah, K. S. E., Griffin, J. R., Pope, F. M., Solomon, E., A highly polymorphic region 3' to the human type II collagen gene. Nuc. Acids Res. 1985, 13: 4613-4620

16. Wahl, W. P., Wallace, L. J., Moore, P. J. A hot spot for homologous recombination in human cells. Cell 1990, 60: 95-103

17. Mathani, M. M., Willard, H. F. A polymorphic X-linked tetranucleotide repeat locus displaying a high rate of new mutation: implications for mechanisms of mutation at short tandem repeat loci. Hum. Mol. Gen. 1993, 2: 413-417

18. Weber, J. L., Wong, C. Mutation of human short tandem repeats. Hum. Mol. Gen. 1993, 2: 1123-1128

19. Levinson, G., Gutman, G. A. Slipped-strand mispairing: a major mechanisms for DNA sequence evolution. Mol. Bio. Evol. 1987, 4:203-221

20. Jeffreys, A. J., Boris, P., Buard, J., Collick, A., Dubrova, Y., Hollies, C. R., May, C. A., Murray, J., Neil, D. L., Neumann, R., Stead, J. D. H., Tamaki, K., Holley, M. Spontaneous and induced minisatellite instability. Electrophoresis 1997, 9:1501-1511

21. Lewin, B. Die evolution der hierarchischen Variationen in der Satelliten DNA. S. 579-582. In: Lewin, B. Gene. 1991, 2 auflage, VHC-Verlag, Weinheim

22. Edwards, A. Hammond, H. Jin. L., Caskey, T., Chakraborty, R. Genetic variation at trimeric tandem repeat loci in four human population groups. Genomics 1992, 12: 241-253

23. Roewer, L., Arnemann, J., Spur, N. K., Grzeschik, K. H., Epplen, J. T. Simple repeat sequence on the Y chromosome are equally polymorphic as their autosomal counterparts. Hum. Gen. 1992, 89:389-394

24. Joblin, M. A., Tyler, S. C. Father and sons: The Y chromosome and human evolution. Trends Genet. 1995, 11: 449-456

25. Kayser, M. Caglia, A. Corach, D. Fretwell, N. Gehrig, C. Graziosi, G. Heidorn, F., Herrmann, S., Herzog, B., Hidding, M., Honda, K., Jobling, M., Leim, K., Meuser, S., Meyer, E., Osterreich, W., Pandya, A., Parson, W., Piccinini, A., Perez-Lezaun, A., Prinz, M., Schmitt, C., Schneider, P. M., Szibor, R., Teifel-Greding, J., Weichhold, G., de Kniff, P., Roewer, L. Evolution of the Y-chromosomal STRs: A Multicenter study. Int. J. Leg. Med. 1997, 110:125-133

26. Roewer, L., Kayser, M., Nagy, M., de Kniff, P. Male identification using Y-chromosomal STR polymorphism. In: Advances in forensic haemogenetics 6. Carracedo, A., Brinkmann, B., Bar, W., Spinger-Verlag, Heidelberg, Newyork. 1996, P 124-126

27. Mathias, N., Bayes, M., Tyler, S. M. C. Highly informative compound haplotypes for the human Y chromosome. Hum. Mol. Gen. 1994, 3:115-123

28. Miller, S. A., Dykes, D. D. Poleskey, H., F. A simple salting out procedure for extract DNA from human nucleated cells. Nucl. Acid Res. 1998, 16: 1215

29. Bio-Rad Laboratories Catalog Bio-Rad Laboratories, Hercules, CA, 1996, p. 85

30. Bio-Rad Laboratories Chelex 100 and Chelex 20 chelating ion exchange resin instruction manual 1996, p. 1-24

31. Saiki, R. K., Scharf, S. J., Faloona, F., Moolis, K. B., Horn, G. T., Frlich, H. A., Arnheim, N. Enzymatic amplification of Beta-Globin genomic sequence and restriction site analysis for diagnosis of single cell anemia. Science 1985, 230: 135-141

32. Don, R. H., Cox, P. T., Wainright, B. J., Baker, K., Matick, J. S. 'Touchdown' PCR to circumvent spurious priming during gene amplification. Nucl. Acids Res. 1991, 19: 4008

33. Sammons, D. W., Adams, L. D., Nishizawe, E. E. Ultrasensitive silver-based color staining of polypeptides in polyacrylamide gels. Electrophoresis 1981, 2:135-141

34. Schneider, P. M., Meuser, S., Waiyawuth, W., Seo, Y., Rittner, C. Tandem repeat structure of the duplicated Y-chromosomal STR locus DYS385 and frequency studies in the German and three Asian popultion. Forensic Sci. Int. 1998, 97: 61-70

35. DNA commission of the ISFH report concerning further recommendations of the DNA commission of the ISFH regarding PCR-based polymorphism in short tandem repeat. Int. J. Legal Med. 1994, 107:159-160

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

37. Casanova, M., Leroy, P., Boucekkine, C., Weissenbach, J., Bishop, C., Fellous, M., Purello, M., Fiori, F., Siniscalco, M. A Y-linked DNA polymorphism and its potential for estimating genetic and evolutionary distance, Science 1985, 230:1403-1406

38. De Kniff, P., Kayser, M., Caglia, A., Corach, D., Fretwell, N., Gehrig, C., Graziosi, G., Heidorn, F., Herrmann, S., Herzog, B., Hidding, M., Honda, K., Jobling, M., Leim, K., Meuser, S., Meyer, E., Osterreich, W., Pandya, A., Parson, W., Piccinini, A., Perez-Lezaun, A., Prinz, M., Schmitt, C., Schneider, P. M., Szibor, R., Teifel-Greding, J., Weichhold, G., Roewer, L. Chromosome Y microsatellite: population genetic and evolutionary aspects. Int. J. Legal. Med. 1997, 110: 134-149

39. Hammer, M. F. A recent insertion of an Alu element on Y chromosome is a useful marker of human population studies. Mol. Bio. Evol. 1994, 11:749-761

40. Zhang, L., Waiyawuth, W., Rittner, C. Schneider, P. M. Population genetics of the Y-chromosomal Alu repeat insertion polymorphism DYS287 (YAP), in; Olaisen, B., et al. (Eds). Advances in forensic Haemogenetics 7, Elsevier, Amsterdam 1998, p.533-535

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