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Allele Frequencies of CSF1PO, TPOX and THO1 STRs in Random Chinese Population of Malaysia

Dissertation submitted in partial fulfilment for the Degree of Bachelor of Science (Health) in Forensic Science

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CERTIFICATE

This is to certify the dissertation entitled

"Allele Frequencies of CSF1PO, TPOX and THO1 in Random Chinese population of Malaysia

is the bonafide record of research work done by

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During the period 8/11/03 to 1/2/04

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Abstract

Allele and genotype distribution pattern for three tetrameric short tandem repeat (STR) loci were established from 100 unrelated ethnic Chinese population of Malaysia using polymerase chain reaction (PCR), followed by electrophoresis of the PCR products in denaturing polyacrylamide gels and subsequent detection by silver staining. The STR loci are CSF1PO, TPOX, and THO1. This study was done to evaluate their significance in human identification and population genetics study for forensic casework analysis. The observed genotypic distribution showed no significant deviation from Hardy-Weinberg equilibrium ($P>0.05$). The data in this study have been compared with other published population data.

Introduction

Deoxyribonucleic acid (DNA) is found in every cell that replicates. Encoded in its structure is all genetic information that forms the basis of life. DNA typing was introduced in the mid-1980s. It has revolutionised forensic science and the ability of law enforcement to link perpetrators with crime scenes. Within the past few years DNA-based methods that were specifically designed and optimised for the use of analysis of the trace biological materials have emerged. Thousands of cases have been closed with innocent suspects freed and the guilty ones punished because of the power of a silent biological witness at the crime scene. With a single drop of blood or single strand of hair, this technology can be used in the criminal justice system for both exclusion and inclusion purposes.

The nature of DNA is genetically determined and therefore unique to an individual. DNA analysis can match an individual with a much higher degree of accuracy, estimated to approach 99% (Alcamo, 1995). Because of its specificity, DNA profiling is also extensively used in paternity testing, where the paternity of the child is in question. Anthropologists and forensic pathologists also use this technique in the study of biology and evolution.

DNA materials are widely used in Forensic testing due to its properties that outweigh all other biological materials. There are two basic principles to apply scientific methodology to forensic diagnostic testing of physical evidence collected at crime scenes: that the

sample is appropriate for the scientific analysis to be used and that the technology of the test is based on sound scientific principles (Herber and Saferstein; 1997). Herber and Saferstein (1997) also elaborated on these two principles to describe six desirable aspects of the DNA marker.

- DNA contains the genetic code for all living cells; with the exception of identical twins, it is believed that the DNA of each individual is different. Thus, the DNA of every human has the potential to be used as a unique identifier of that person.
- Every cell contains a complete copy of the DNA of the individual from whom it is obtained. Thus, any cellular material from an individual potentially yields the same identifier, making it possible to compare different types of biological sample, such as blood, saliva and semen.
- DNA is a robust molecule. It is able to survive physical denaturation as well as being relatively resistant to natural catabolic processes. Thus, any cellular sample that has been stored under conditions of maintenance favourable to the preservation of the structure of DNA can potentially be analyzed successfully.
- The analytical procedure for DNA typing is relatively simple and straightforward. The whole process takes a mere 3 days to complete.
- The analytical sensitivity achievable based on analysis of DNA is high. Current molecular biology methods allow for analysis of a sample that contains as few as 100 molecules of DNA, meaning that for markers where there is a single copy gene per cell, as few as 100 cells need to be analysed.

- DNA contains the genetic code; every cell contains a complete copy of this code. Thus, all polymorphic genetic systems are potentially suitable targets for genetic typing in a forensic sample, as long as the forensic sample contains cellular material or DNA molecules (Herber and Safferstein; 1997).

Short Tandem Repeat (STR) based DNA typing is the state of the art technology widely used in most of the Forensic Science laboratories in the world. STRs are short sequences of DNA, normally of length 2-7 base pairs, that are repeated many times in a head-tail manner. The polymorphisms in STRs are due to the different number of copies of the repeat element that can occur in a population of individuals. The STR loci are found on almost every chromosome in the genome.

Literature Review

Identification and individualisation of evidence materials is the primary task of forensic scientists around the world. Forensic scientists have been using polymorphic genetic markers, such as blood groups and serum proteins, which are inherited in simple Mendelian way (Race and Sanger, 1952). In his paper, Mendel (1866) investigated 'traits' passed from parents to progeny and coined the term dominant and recessive traits (Mendel, 1866). Due to systematic evaluation and documentation of these polymorphic genetic markers to characterize and individualise forensic human biospecimens, more information has been gathered about these antigens (Race and Sanger, 1952; Sanger et al., 1952; Kirk et al., 1962; Prokop and Uhlenbruk, 1965; Giblett, 1969; Kostaszuk et al., 1974; Salzano at al, 1974; Race and Sanger, 1975; Walter et al., 1975; Need et al., 1977a; Naidu et al., 1978; Grunbaum et al., 1980; Norrgard et al., 1984; Salzano et al., 1985; Yung et al., 1989; Lee, 1989; Lee, 1997 and Tournamille et al., 1995). Forensic DNA typing or DNA profiling (Alec Jeffreys et al., 1985) has entered in routine use in forensic science institutions making obsolete the use of blood groups and other polymorphic biochemical genetic markers. All living organisms are made up of cells, the basic integrated units of biological activity. In humans, as in other higher organisms, the hereditary material (Oswald Avery, 1944) deoxyribonucleic acid, or DNA (Watson and Crick, 1953) is contained in the cell nucleus in microscopic assemblages known as chromosomes (Peter, 1959). Human cells have 23 pairs of chromosomes for a total of 46. A typical human cell has one pair of sex chromosomes, and 22 pairs of non-sex chromosomes, known as autosomes. An individual will receive a pair of chromosomes

from each of their parents. Chromosomes are numbered based on their size, the largest being chromosome 1. The units of inheritance are known as genes. Sutton (1902) observed chromosomal movement during meiosis during meiosis and developed the chromosomal theory of heredity. Human beings are thought to have 50,000 - 100,000 genes which are contained in the chromosomal DNA in the cell nucleus. The science of forensic DNA analysis, also called DNA fingerprinting, is little more than a decade old. DNA is a complex double-chained molecule twisted into a helical form: the famous "double helix" structure (Watson and Crick, 1953). The structure of DNA resembles a spiral ladder whereby the "sides" are made of sugar-phosphate molecules and the "rungs" are formed from bases or nucleotides (Chargaff et al., 1949). In DNA from all sources there are only four bases: adenine, thymine, guanine and cytosine. The base pairing is specific, that is, adenine is always paired with thymine, and cytosine is always paired with guanine (Chargaff et al., 1949). This is called "Chargaff's Rules". This specificity of base-pairing also provides a mechanism through which "parent" DNA molecules can be copied to form identical "daughter" DNA molecules in the process of reproduction. Marshall Nirenberg (1963) and H. Gobind Khorana (1966) lead teams that cracked the genetic code. They demonstrated that each of 20 amino acids is coded by a sequence of three nucleotide base. The mechanism, known as replication, is possible because the two sides of the parent DNA molecule are complementary to each other. During this process, the parent DNA molecule "splits" along its length, each side serving as a template for one of the new and identical daughter molecules.

Although all cells contain DNA, only a small fraction of that DNA is actually used to form genes. Most of the DNA in our chromosomes has no known function; the portion of such DNA may be more than 95% of the total complement in humans. The presence of so much "junk DNA" is ironically, however, that is of special interest to the forensic scientist. This non-coding DNA contains repeated base-pair sequences arranged in tandem which are inherited by the individual from his or her parents just as functional genes are inherited. Coding DNA also contains such repeats, but less commonly than non-coding DNA. These tandem repeats combined make up a molecular DNA "fingerprint" (Jeffreys et al., 1985) that is believed to be unique for each individual (with the possible exception of identical twins) because the number of repeated sequences can vary from person to person. These non-coding base-pair repeated sequences are called variable number tandem repeats, abbreviated to VNTR (Nakamura et al., 1987). David Botstein and others (1980) discovered a very useful type of DNA polymorphism, called restriction fragment length polymorphisms (RFLPs). RFLPs are found throughout the genome and are extremely valuable as genetic markers in human genetic studies (Jeffreys et al., 1985).

The polymerase chain reaction, or PCR, is an innovative technique for increasing the amount of a specific sequence of DNA in a sample (Mullis et al., 1980). Alec Jeffreys et al. (1985) introduced DNA fingerprinting using minisatellite based RFLP technique as a method of identification. Many RFLP loci useful for use in paternity testing and personal identification were widely documented (Capon et al., 1983; 1983 Jeffreys et al., 1985; Clark, 1987; Delvin et al., 1990; Goodbuorn et al.,). Population data base on many RFLP

polymorphism is also well documented in the literature (Baird et al., 1986; Long et al., 1986a; Long et al., 1986b; Balazs et al., 1989; Chimera et al., 1989; Deka R, 1991; Kidd et al., 1991) Short tandem repeats, as the name implies, are similar to VNTRs, except that the repeated units are much shorter ranging from 2 to 7 base pairs in length (Edwards et al., 1991 and 1992; and Warne et al., 1991). STRs are found widely distributed in the human genome, are highly polymorphic and can be detected using PCR (Sambrook et al., 1989; Erlich, 1989; Innis et al., 1990 and Ausubel et al., 1993). STR typing can be done with degraded forensic biospecimens and the amplification products are less than 400 base pairs (Nakamura et al., 1987). Published population data base for many different populations are available (Edwards et al., 1991; Polymeropoulous et al., 1992; Budowle et al., 1994; Nellemann, 1994; Pfitzinger, 1995; Andres, 1996; Nagai et al., 1996; Lorente et al., 1997; Garofano et al., 1998; Pu et al., 1998; Sinha et al., 1999; Rahman, 2000; Han, 2001; Bagnodavicious et al., 2002 and Dobashi et al., 2003). Published data base on STRs for ethnic population groups in Malaysia is very few (Seah et al., 2003 and Panneerchelvam et al., 2003). Hence in the present study allelic distribution for CSF1PO, TPOX and TH01 STRs for Chinese ethnic group of Malaysia were studied.

OBJECTIVES OF THE STUDY

The objectives of the study are:

1. To develop a population database by compiling the distribution patterns of various alleles for 3 STRs which are CSF1PO, TPOX and THO1 in random Chinese population. This database can be used in any forensic science institution around the world.
2. To compare the studied data base with the data bases of other populations in the world.

MATERIALS AND METHODS

1.0 Materials

All materials and reagents used in this study are sterile. The methods were followed according to the GenePrint STR System Technical Manual.

1.1 Sample source

Buccal swabs were taken from 100 healthy unrelated random Chinese individuals within Malaysia. Sterile cotton buds were used and each individual was asked to streak the cotton buds from inside their mouth towards cheek at least 10 times. The cotton buds were air dried at room temperature away from direct sunlight and kept in a transparent plastic wrapper. All samples were labelled with name, father's name, place of birth and gender.

1.2 Reagents

Digestion buffer (1M Tris HCL pH 7.5 , 0.5M EDTA , 20% SDS , 5M NaCl), Proteinase K (20μg/μl) (Promega, USA), Chloroform -isoamyl (24:1), 3M sodium acetate, 2M sodium acetate, 70% ethanol, TE buffer, 10% NaOH, 0.5% acetic acid in ethanol, bind saline (Promega, USA), 10% ammonium persulphate, 40% acrylamide, 10X TBE buffer, 0.5X TBE buffer, CTT Multiplex kit [STR 10X buffer (500mM KCl, 100mM Tris-HCl (pH 9), 15mM $MgCl_2$, 1% Triton X-100, 2mM each dNTP), multiplex 10X CTT primer pair mix, Taq DNA polymerase (5μ/μl), STR 2X Loading Solution (10mM NaOH, 95% formamide, 0.05% bromophenol blue, 0.05% xylene cyanol FF), CTT Allelic Ladder

Mix] (Promega, USA), acrylamide solution, silver nitrate staining solution, fix/stop solution, gel developer, Rain X (Blue Coral-Slick 50, USA).

1.3 Chemicals

Tris base (Promega, USA), concentrated HCL, Na_2EDTA (Promega, USA), NaOH (Merck, Germany), NaCl (Merck, Germany), sodium dodecyl sulphate (SDS) (Bio-Rad Lab.), chloroform (Merck, Germany), isoamyl (Merck, Germany), sodium acetate (Merck, Germany), glacial acetic acid (Merck, Germany), absolute ethanol (Merck, Germany), EDTA (Promega, USA), ammonium persulphate (Promega, USA), acrylamide (Promega, USA), bisacrylamide (Promega, USA), boric acid (Promega, USA), urea (Promega, USA), silver nitrate (Promega, USA), 37% formaldehyde (Promega, USA), sodium thiosulphate (Promega, USA), sodium carbonate (Promega, USA) and TEMED (N,N N'N' tetramethylethylene diamine)(Promega, USA), phenol (PIERCE, USA).

1.4 Apparatus

Desiccators Nucerite (Nalge/Syborn Corp), vortex mix EVM-6000 (ERLA), spectrafuge 16M (National Labnet Co.), Gilson automated pipette (1000µl, 200µl, 100µl, 20µl and 10µl), parafilm (American National Can), MJ Research PTC-200 Peltier Thermal Cycler (Gradient cycler) used for 0.2µl tubes, MJ Research PTC-100 Peltier Thermal Cycler used for 0.5µl tubes, DRAGON 204 Mettler Toledo weighing machine, Fisher & Pagkal N308 Freezer, Hitachi refrigerator, Mammart waterbath, GIBCO BRL Sequencing

System, EC 3000-90 power pack, fumehood (Model : RICO), trays for staining, stopwatch.

2.0 Reagent Preparation and Methodology

2.1 DNA Extraction

2.1.1 Reagent preparation

1M Tris HCL pH 7.5

121.1g Tris base was dissolved in 800ml deionised water and the pH adjusted to 7.5 with concentrated HCl. Solution was made up to 1000ml and autoclaved.

0.5M EDTA

186.1g Na_2EDTA was dissolved in 800ml deionised water and the pH adjusted to 8.0 with NaOH pellets. The solution was made up to 1000ml and autoclaved.

5M NaCl

292.2g NaCl was added to 800ml deionised water and made up to 1000ml.

20% SDS

An empty bottle was autoclaved. 100g Sodium dodecyl sulphate was added to 400ml deionised water. Solution was stirred with heat using magnetic stirrer. Make up to 500ml.

Digestion buffer (Do not autoclave)

1ml of 1M Tris HCL pH7.5, 2ml of 0.5M EDTA, 10ml of 20% SDS, 1ml of 5M NaCl and 86ml of deionised water were mixed together.

Proteinase K (20µg/µl)

20 mg of Proteinase K was mixed with 1ml of deionised water.

Chloroform - isoamyl alcohol (24:1)

240ml of chloroform was added to 10ml of isoamyl alcohol.

3M sodium acetate

102.025g of sodium acetate was added to 200ml of deionised water. The pH was adjusted to 5.2 with glacial acetic acid. Solution was made up to 250ml and autoclaved.

2M sodium acetate

16ml of 3M sodium acetate was added to 8ml of deionised water.

70 % ethanol

350ml of absolute ethanol was mixed with 150ml deionised water.

TE buffer

10ml of 1M Tris HCL was mixed with 0.2ml 0.5M EDTA. 989.8ml of deionised water was added and autoclaved.

2.1.2 Extraction Method

The cotton swabs were cut into small pieces and placed into a 1.5ml tube. 500µl of digestion buffer and 12µl Protease K (20mg/ml) were added into the sample and incubated overnight at 56°C. 120µl buffered phenol was added and mixed vigorously using the vortex. The samples were centrifuged at 10,000rpm for 3 minutes. By using cut tips, the supernatant was transferred to another 1.5 ml tube. To this, 1 volume (250µl) of

buffered phenol and chloroform- isoamyl was added. The contents of the micro centrifuge tube was mixed vigorously and again centrifuged at 10,000rpm for 3 minutes. Using cut tips, the supernatant was again transferred to a new tube. 1 volume Chloroform –isoamyl (same volume as supernatant) was added and mixed vigorously. Samples were centrifuged at 10,000rpm for 5 minutes. Supernatant was transferred to a new tube using cut tips. 500µl chilled ethanol and 50µl 2M sodium acetate were added to the sample. The sample was mixed by inverting the tube slowly. The sample was then centrifuged for 10 minutes at 10,000rpm. The supernatant was then discarded and 0.5ml 70% ethanol was added. DNA pellet was dislodged and again centrifuged at 10,000rpm for 3 minutes. Supernatant was discarded. The tubes were sealed with parafilm and the pellets were dried using vacuum pump. On drying, 30µl TE Buffer was added and kept overnight at 37°C. The samples were kept in -20°C for further usage.

2.2 DNA Quantification

2.2.1 Reagent preparations

Ethidium bromide stock solution

1g of ethidium bromide was dissolved in 100ml of deionised water and stored in amber bottle.

1% agarose gel solution

1g agarose powder was dissolved in 100ml 0.5X TBE. 0.07µl of ethidium bromide stock solution was added to the agarose solution.

2.2.2 Preparatory agarose gel electrophoresis and spectrophotometry

The presence of DNA was detected from the extracted samples by using preparatory agarose gel electrophoresis. 5µl of DNA sample was used for observing the presence of DNA. An example of the agarose electrophoregram is shown in Figure 1. The DNA samples were quantified using spectrophotometer at 260nm. DNA samples were appropriately diluted, so that 1µl of DNA sample consists of 10ng of DNA.

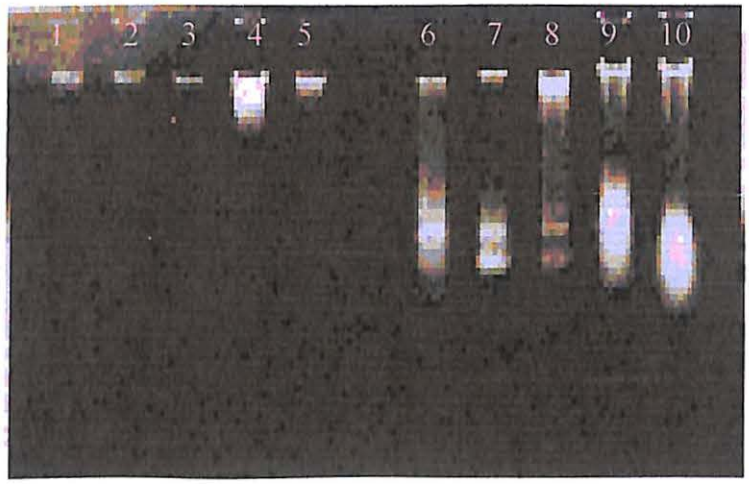

Figure 1. An electrophoregram of DNA samples. Samples in lanes 1 to 10 all contain DNA at different concentration.

2.3 PCR Amplification

The amplification of HUMTHO1, TPOX and CSF1PO STR loci was performed using multiplex primer kit according to the manufacturer's instructions (Promega Corporation, USA). The STR 10X Buffer and STR 10X CTT primer pairs were thawed and kept on ice. The number of reactions to be set up was determined. 1 or 2 reactions were added to

this number to compensate for pipetting error. The required amount for each component of the PCR Master Mix was calculated (Table 1). The volume per sample was multiplied by the total number of reactions to obtain the final volume. The volume for each component was added to a sterile tube.

Table 1: PCR Master Mix Component

PCR Master mix component	**Volume per sample (µl)**
Sterile water	17.35
STR 10X Buffer	2.50
CTT Multiplex 10X Primer Pair Mix	2.50
Taq DNA Polymerase (5u/µl)	0.15 (0.75u)
Total volume	**22.50**

22.5µl of PCR master mix was added to each tube and placed on ice. 2.5µl of sample was pipetted into the respective tubes containing 22.2µl of the PCR master mix. The samples were then centrifuged briefly to bring the contents to the bottom of the tube. The recommended protocol was used to run the amplification (Table 2).

Table 2. Amplification protocol for CTT Multiplex

Initial incubation	**Cycling for first 10 cycles**	**Cycling for last 20 cycles**	**Hold step**
96°C for 2 minutes	94°C, 1 minute 64°C, 1 minute 70°C, 1.5 minute	90°C, 1 minute 64°C, 1 minute 70°C, 1.5 minute	4°C

The primers for the STRs that were used are as follows (Huang *et al.*, 1995):

HUMTHO1	5' GTG GGC TGA AAA GCT CCC GAT TAT 3'	(forward)
HUMTHO1	5' ATT CAA AGG GTA TCT GGG CTC TGG 3'	(reverse)
TPOX	5' ACT GGC ACA GAA CAG GCA CTT AGG 3'	(forward)
TPOX	5' GGA GGA ACT GGG AAC CAC ACA GGT 3'	(reverse)
CSF1PO	5' AAC CTG AGT CTG CCA AGG ACT AGC 3'	(forward)
CSF1PO	5' TTC CAC ACA CCA CTG GCC ATC TTC 3'	(reverse)

The tubes were then placed in the thermal cycler for amplification. A picture of the thermal cycler is shown in Figure 2.

Figure 2. Thermal cycler

2.4 Electrophoresis

2.4.1 Reagent preparation

10 % NaOH

100g of NaOH pellets were dissolved in 1000ml of deionised water.

0.5 % acetic acid in ethanol

0.25ml acetic acid was added to 49.75ml absolute ethanol in a 50ml centrifuge tube.

Bind silane

3µl bind silane (silver stain kit) was added to 1ml of 0.5% acetic acid in ethanol in a 2ml micro centrifuge tube.

20 % ammonium persulphate

0.2g of ammonium persulphate (AP) was added to 1ml deionised water. Solution must be freshly prepared.

40 % acrylamide: bisacrylamide (19:1)

190.0g of acrylamide and 10.0g of bisacrylamide were dissolved in 250ml of deionised water. Solution was made up to 500ml and kept chilled. Solution was placed in amber bottle.

10X TBE buffer

107.8g of Tris base and 7.44g of Na_2EDTA were added together with 800ml of deionised water. 46.0g boric acid was added slowly. The pH was adjusted to 8.3 by adding extra 9.0g of boric acid. Solution was made up to 1000ml and autoclaved.

0.5X TBE buffer

50 ml 10X TBE buffer was added to 950ml deionised water.

6% Acrylamide gel solution

25.2g of urea, 3.0ml of 10X TBE buffer, 9ml of 40% acrylamide and 29ml of deionised water were mixed together in a 100ml beaker and stirred until fully dissolved (Table 3).

Table 3. Components of acrylamide solution

Components	6% gel concentration
Urea	25.2g
Deionised water	29.0 ml
10X TBE	3.0 ml
40% acrylamide (19:1)	9.0 ml
Total	**60 ml**

2.4.2 Electrophoresis methodology

A long and a short glass plates were used. Both plates were cleaned with 95% ethanol and with tissues. 3ml of Rain-X was applied to the longer plate. The solution was spread all over the plate with a dry paper towel using circular motion. In the meantime, fresh bind silane was prepared and then poured on the short glass plate. The short glass plate was wiped with a tissue using circular motion. On drying, the long glass plate was wiped with deionised water, whereas the short glass plate was wiped with 95% ethanol. A pair of 0.4mm spacers was placed on each side of the long plate. Both the glass plates were assembled and sealed with sealing tape. A 6% acrylamide solution was prepared and

filtered using the 0.2 μ filter. After preparation of the above, TEMED and 20% ammonium persulphate were added. Using a 50cc syringe with 21 gauge needle, the acrylamide solution was poured between the glass plates. The gel was positioned in a slant and the straight side of the sharktooth comb was inserted. The gel was left for polymerization for two hours.

The sealing tapes were removed and the outside of the glass plates were cleaned. 0.5X TBE buffer was added to the bottom chamber of the electrophoresis apparatus. The glass plates were placed in the electrophoresis apparatus, long plate facing out. The glass plates were secured with the electrophoresis apparatus by means of clamps provided. 0.5X TBE buffer was added to the top buffer chamber. Air bubbles on the top of the gel were removed and the apparatus was then assembled for prerun for half an hour at 40 watts.

Meanwhile, the PCR products were prepared for loading. 2μl of STR 2X loading solution and 3μl of sample were mixed in a tube. 2μl of STR 2X loading solution and 3μl of allelic ladder sample provided was also prepared. The samples and the ladders were denatured at 95°C for 2 minutes prior to loading. The samples and the ladders were placed on ice immediately after denaturing.

After the prerun, the buffer was flushed again with syringe. The sharktooth comb was inserted into the gel. 4μl of the sample and the allelic ladders were loaded into the wells. Loading of the samples should not take more than 20 minutes. The gel was run at 40 watts until the dye reached almost the bottom of the plate. The plates were removed from

the electrophoresis apparatus and the short glass plate, with the gel attached, was stained. A picture of the electrophoresis apparatus is shown in Figure 3.

Figure. 3: GIBCO BRL Sequencing system

2.5 Silver Staining

2.5.1 Reagent preparations for staining

Silver nitrate solution

0.6g of silver nitrate and 0.9ml of 37% formaldehyde were mixed in a beaker. 600ml of deionised water was added and the beaker was wrapped with aluminium foil. The solution was kept at 4 °C.

Fix/stop solution

120ml of glacial acetic acid was added to 480 ml of deionised water.

Gel developer

0.6ml of 37% formaldehyde was added to 120µl sodium thiosulphate and 18g of sodium carbonate in a beaker. Solution was made up to 600ml using deionised water.

2.5.2 Silver Staining Methodology

The gel bands can be detected by silver staining method as per the instructions in the Promega's DNA Silver Staining System technical manual of the manufacturer (Promega, USA). The shorter glass plate with gel were placed into the fix or stop solution for 20 minutes. Then, it was washed with deionised water for 2 minutes. The washing was repeated for another 2 minutes with new deionised water. The gel was stained for 30 minutes in the silver staining solution. Another 10 seconds washing was done with deionised water, just after the staining. The gel was then placed into the developer solution up to 5 minutes, until the alleles and ladders were visible. The developed gel was then fixed with the stop solution for 5 minutes. Finally, the gel was washed in deionised water for 2 minutes. The shorter plate was dried in upright position. The results were recorded in a table. The protocols relating to silver staining is shown in table 4.

Table 4. Procedures of silver staining as in the Promega's DNA Silver Staining System

Step	Solution	Time
I	Fix/stop solution	20 minutes
II	Deionised water	2 minutes
III	Repeat step II, twice	2 x 2 minutes
IV	Staining solution	30 minutes
V	Deionised water	10 seconds
VI	Developer solution (4-10°C)	Up to 5 minutes (until alleles and ladders are visible)
VII	Fix/stop solution	5 minutes
VIII	Deionised water	2 minutes

RESULTS

The genotypes on the three STR's- CSF1PO, THO1 and TPOX of the random Chinese individuals were recorded and the genotype frequencies for each STR were estimated by the widely used method of maximum likelihood. An example of the STR result is shown in figure 4. The formula of maximum likelihood for estimating gene frequencies for two allelic (p1 and p2) co dominant system is:

$$p1 = 2x + y/2N \qquad \text{and}$$

$$p2 = 1 - p1$$

'x' symbolises the number of homozygous type, 'y' symbolises the number of heterozygous type, and 'N' is the total number of individuals analysed. Gene frequencies were computed and Chi-squared test was also performed to assess the randomness of the population taken for this study. The results are shown in tables 5, 6, 7 and 8. The allele frequencies of the three STRs were compared with other population groups (Tables 9-28). There was no deviation from the Hardy-Weinberg equilibrium. The heterozygosity for STRs CSF1PO, TPOX and THO1 are 87.31%, 83.66% and 78.61% respectively (Table 8). The power of discrimination (PD value Table 8) is 92.69%, 79.63% and 82.62% respectively. The cumulative power of discrimination of the three STRs is 0.9985.