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**Allele frequencies of STRs (CSF1PO, TPOX and TH01) in
random Iban population of Eastern Malaysia**

**Dissertation submitted in partial fulfillment for the Degree of
Bachelor of Science in Forensic Science**

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2006

CERTIFICATE

This is to certify that the dissertation entitled

**“Allele frequencies of STRs (CSF1PO, TPOX and TH01) in
random Iban population of Eastern Malaysia”**

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CONTENTS

| | |
|-----------------------|----|
| ABSTRACT | 1 |
| INTRODUCTION | 2 |
| LITERATURE REVIEW | 6 |
| OBJECTIVE OF STUDY | 11 |
| MATERIALS AND METHODS | 12 |
| RESULT | 28 |
| DISCUSSION | 32 |
| CONCLUSION | 49 |
| REFERENCES | 50 |

LIST OF TABLES

| | | |
|----------|--|----|
| Table 1 | Chemicals and reagents used in the study | 14 |
| Table 2 | Amplification materials used in the study | 14 |
| Table 3 | Laboratory equipments used in the study | 15 |
| Table 4 | The components of Master Mix used in amplification setup | 20 |
| Table 5 | Thermal Cycling Condition | 21 |
| Table 6 | The components of 4% or 6% acrylamide solution | 23 |
| Table 7 | Silver staining method | 27 |
| Table 8 | Details that showing the calculation relating to randomness of the samples (CSF1PO) Hardy-Weinberg equilibrium | 29 |
| Table 9 | Details that showing the calculation relating to randomness of the samples (TPOX) Hardy-Weinberg equilibrium | 29 |
| Table 10 | Details that showing the calculation relating to randomness of the samples (TH01) Hardy-Weinberg equilibrium | 30 |
| Table 11 | Allele frequencies of CSF1PO, TPOX and TH01 loci in Iban population (n=100) | 31 |
| Table 12 | The distribution of allele frequencies for CSF1PO STR in Chinese, Japanese, Thailand (Taiwan), Thai and Philippines Population | 36 |
| Table 13 | The distribution of allele frequencies for CSF1PO STR in Chinese (Hong Kong), Baniya, Chakkiliyar, Gope and Gounder Population | 36 |
| Table 14 | The distribution of allele frequencies for CSF1PO STR in Irular, Karan, Khandayat, Kurmi and Oriya Brahmin Population | 36 |

| | | |
|----------|---|----|
| Table 15 | The distribution of allele frequencies for CSF1PO STR in Visayas, Yadav, Hmar, Koreans and Lai Population | 37 |
| Table 16 | The distribution of allele frequencies for CSF1PO STR in Lusei, Malay, Mara, Chinese and Sala Population | 37 |
| Table 17 | The distribution of allele frequencies for CSF1PO STR in Tu, Aragon, Maine, Swiss Caucasian and African American Population | 37 |
| Table 18 | The distribution of allele frequencies for CSF1PO STR in US Caucasian, Italian, Scottish, Ecuador and Turkey Population | 38 |
| Table 19 | The distribution of allele frequencies for CSF1PO STR in Turkish, African American, Columbian, Cordoba and Hispanic Population | 38 |
| Table 20 | The distribution of allele frequencies for CSF1PO STR in Mestizos, Moroccan, Northeastern Poland, Paraguay and Rio Grande do Norte Population | 38 |
| Table 21 | The distribution of allele frequencies for CSF1PO STR in Santa Catarina, Turkish, US Caucasian, Brazilian Caucasian and Brazilian (Northeast Brazil) Population | 39 |
| Table 22 | The distribution of allele frequencies for CSF1PO STR in European-descendant, Lithuanians and Northern Polish Population | 39 |
| Table 23 | The distribution of allele frequencies for TPOX STR in Chinese, Japanese, Thailand (Taiwan), Thai and Philippines Population | 40 |
| Table 24 | The distribution of allele frequencies for TPOX STR in Chinese (Hong Kong), Baniya, Chakkiliyar, Gope and Gounder Population | 40 |
| Table 25 | The distribution of allele frequencies TPOX STR in Irular, Karan, Khandayat, Kurmi and Oriya Brahmin Population | 40 |
| Table 26 | The distribution of allele frequencies for TPOX STR in Turkey, Turkish (Kosovo), Visayas, Yadav and Hmar Population | 41 |
| Table 27 | The distribution of allele frequencies for TPOX STR in Lai, Lusei, Malay, Mara and Turkish Population | 41 |

| | | |
|----------|---|----|
| Table 28 | The distribution of allele frequencies for TPOX STR in Chinese, Sala, Tu, Aragon and Asturias Population | 41 |
| Table 29 | The distribution of allele frequencies for TPOX STR in Maine, Swiss Caucasian, African American, US Caucasian and Italian Population | 42 |
| Table 30 | The distribution of allele frequencies for TPOX STR in Italian, Scottish, Ecuador, African American and Colombian Population | 42 |
| Table 31 | The distribution of allele frequencies for TPOX STR in Cordoba, Hispanic, Mestizos, Moroccan and Poland Population | 42 |
| Table 32 | The distribution of allele frequencies for TPOX STR in Paraguay, Rio Grande do Norte, Santa Catarina, US Caucasian and Brazilian Population | 43 |
| Table 33 | The distribution of allele frequencies for TPOX STR in Brazilian, Costa Rico, Lithuanian, North Polish and European Descendants (Brazil) Population | 43 |
| Table 34 | The distribution of allele frequencies for TH01 STR in Chinese, Japanese, Thailand (Taiwan), Thai and Philippines Population | 44 |
| Table 35 | The distribution of allele frequencies for TH01 STR in Chinese (Hong Kong), Baniya, Chakkiliyar, Gope and Gounder Population | 44 |
| Table 36 | The distribution of allele frequencies for TH01 STR in Irular, Karan, Khandayat, Kurmi and Oriya Brahmin Population | 44 |
| Table 37 | The distribution of allele frequencies for TH01 STR in Turkey, Turkish (Kosovo), Visayas, Yadav and Hmar Population | 45 |
| Table 38 | The distribution of allele frequencies for TH01 STR in Koreans, Lai, Lusei, Malay and Mara Population | 45 |
| Table 39 | The distribution of allele frequencies for TH01 STR in Turkish, Chinese, Sala, Tu and Aragon Population | 45 |
| Table 40 | The distribution of allele frequencies for TH01 STR in Asturias, Maine, Swiss Caucasian, African American and US Caucasian Population | 46 |

| | | |
|----------|---|----|
| Table 41 | The distribution of allele frequencies for TH01 STR in Italian, Scottish, Italy, Ecuador and Central Poland Population | 46 |
| Table 42 | The distribution of allele frequencies for TH01 STR in Aegean Region, Central Poland, S. W. Spain, West African Black Immigrant and Portugal Population | 46 |
| Table 43 | The distribution of allele frequencies for TH01 STR in Moroccan Immigrant, North African, African American, Columbian and Cordoba Population | 47 |
| Table 44 | The distribution of allele frequencies for TH01 STR in Hispanic, Mestizos, Moroccan, Northeastern Poland and Paraguayan Population | 47 |
| Table 45 | The distribution of allele frequencies for TH01 STR in Rio Grande do Norte, Santa Catarina, US Caucasian, Brazilian Caucasian and Brazilian Population | 47 |
| Table 46 | The distribution of allele frequencies for TH01 STR in Lithuanians, Northern Polish and European-descendant (Brazil) Population | 48 |

LISTS OF FIGURES

| | | |
|----------|--|----|
| Figure 1 | Schematic representation of analytical protocol | 12 |
| Figure 2 | Thermal Cycler PTC – 200 | 21 |
| Figure 3 | Electrophoregram of STRs – CSF1PO, TPOX and TH01 | 27 |

ABSTRACT

Advances in DNA technology and the discovery of DNA polymorphisms have led to creation of DNA databases using the microsatellites or short tandem repeat (STRs). The main purpose of creating the DNA database is to study the polymorphism of DNA segments among individuals within the same population and among different human populations. Individuals may have different combinations of the variants and populations will have different frequencies of those variants. These levels of variation in DNA sequence are useful in human identification purpose. In the present study, the distribution of allele frequencies for three validated STRs – CSF1PO, TPOX and TH01 was compiled for the ethnic Iban population of Eastern Malaysia. DNA samples from 100 random Iban individuals were processed using multiplex primer kit. The distributions of allele frequencies of Iban population are unique.

INTRODUCTION

Deoxyribonucleic acid (DNA) analysis led to the release of Stephan Cowan, the 141st person exonerated after he spent six years in prison for a shooting that he did not commit. His conviction was based on fingerprint evidence. Fingerprint evidence was considered as absolute or positive evidence. Fingerprint is used as a power tool for the conviction, but sometimes, fingerprint evidence can lead to wrongful conviction (Cole, 2004).

DNA exonerations have exposed a lot of bad serology examinations. In this case, three armed men break into a home. Four inmates, two males and two females, all are related to each other. Both females were sexually assaulted and the males were beaten with a pistol. One male who lived in the home forcibly obtained a weapon from an assailant and fired. The robbers fled the scene. There were seven individuals who might leave the blood at the scene, three robbers and four victims. Conventional serological testing, the test without DNA analysis, might be able to distinguish between the satins from the seven individuals. If some of them are related, the conventional serological testing might not distinguish among them (Eckert, 1997). The circumstances of this case and also Stephan Cowan's case showed the power of DNA-based identification technology.

DNA is a chemical that controls the inheritance of hair color, eye color, stature, bone density and many other human traits. Our body's cells such as muscle cells, brain cells, liver cells, blood cells, sperm cells and others contain DNA. Basically, every part of the body is made up of tiny cells and each cell contains a complement of DNA identical to that of every other cell within a given person. Every human cell, except for sperm and

eggs, contains more than 7 billion pairs of nucleotides (base pairs) (Friedman, 1999). Analyzing the entire sequences of genomic DNA is time consuming and expensive, so forensic scientists will focus on particular genetic sequences, known as “markers”. These markers are highly variable between individual due to its different arrangement of sequence and they are unique to particular individual. The analysis of a set of DNA genetic markers for individualization is known as DNA profiling or DNA fingerprinting. DNA typing is also applied on other tasks such as determining the compatibility of tissue types in organ transplants, detecting the presence of a specific microorganism, tracking desirable genes in plant breeding, and establishing paternity.

DNA profiling can make use of any specimen that contain DNA such as bloodstains, semen, bone marrow, or other tissue or bodily fluid that has nucleated cells (Curran, 1997). DNA profiling was first used in 1986 in England to identify Colin Pitchfork in the rape and murder of two young girls. The method used in that case was Restriction Fragment Length Polymorphism (RFLP). Although RFLP is a powerful method of identification, but it has few disadvantages such as: RFLP requires intact DNA. Exposure of DNA to sunlight, damp condition can cause DNA degradation. Improper collection and storage of specimen can render the DNA useless for RFLP analysis (Friedman, 1999). The sizes of fragments are not distinct. It is not possible to distinguish between two fragments that are close in size. RFLP test requires 4 to 6 weeks. However, test results can sometimes take 3 to 6 months due to laboratory backlogs.

The polymerase chain reaction (PCR) based on microsatellites or short tandem repeat (STR) analysis has replaced RFLP technique in forensic human identification. PCR method has a number of advantages in forensic DNA testing such as: PCR is very

sensitive. Samples that contain quantities of DNA that are too small for RFLP analysis can often be tested using PCR. PCR works with partial degraded DNA. The DNA in forensic samples is often degraded due to bacterial decomposition and environmental exposure. RFLP require large amount of DNA, and small evidence samples are often consumed during testing. PCR only require small amount of samples. Therefore, a portion of the original evidence can be preserved for independent testing or for future testing. PCR is fast if compared with RFLP (Friedman, 1999).

The STR loci are polymorphic markers consist of variable number of tandem repeat, ranging from two to six nucleotides per locus (Sacchetti *et al*, 1999). STRs are similar to RFLP in that the different size variants can be detected by gel electrophoresis. However, STR variant are of discrete size, whereas RFLP variants are continuously variable. There were several methods can be used to separate STR. The most common method is polyacrylamide gel electrophoresis (Friedman, 1999). The detection of DNA on polyacrylamide gel is by using the silver nitrate staining.

An alternative method of detection uses fluorescently labeled STRs. Fluorochrome-labeled primers are incorporated during the PCR amplification process. After separation through the polyacrylamide gel electrophoresis, the fluorescent scanner is used to scan the labeled STR. Capillary electrophoresis is a new method of STR separation. The DNA is separated by electrophoresis through narrow capillary tube. By coupling with automatic injectors and multiple wavelength fluorescent detection, capillary electrophoresis has a throughput of one multiplexed sample every 15 to 20 minutes (Friedman, 1999).

In 1986, Dawn Ashworth was murdered in Leicestershire, UK. The police involved in investigating this murder and another seemingly linked murder in 1983, decided to use the new technique of DNA profiling in their investigation. The police created an informal DNA database by collecting blood samples from all the males in the town where Dawn Ashworth lived. This was the first use of a 'DNA database' to solve a crime (Stringer, 2002).

The widespread acceptance of DNA typing or DNA profiling by court systems around the world has led many countries to pass laws requiring people who convicted of sex offenses and other crimes to be DNA typed and for creation of a DNA database for the criminals. With this DNA database, it will allow investigators make a rapid comparison and matching of known offenders with genetic material recovered from crime scenes (Guillen *et al*, 2000). The DNA database on each of the ethnic population group is essential to ascertain the distribution of various alleles in that population. The distribution of alleles for each STR locus is imperative for calculation of match probability and in case of paternity testing.

The Iban population in Eastern Malaysia (Sarawak) has been chosen for this project because of this population has not been studied for STR typing yet in Malaysia. The main objective of this project is to study the pattern and distribution of 3 validated used STR loci in the Iban population of Malaysia in order to establish a database that is relevant enough to be referred. The 3 STR loci (CSF1PO, TPOX and TH01) had been chosen for this project.

LITERATURE REVIEW

In 1953, James Watson and Francis Crick discovered the structure of the DNA. Before that, the biologist had difficulty in accepting DNA as the genetic material because of the apparent simplicity of its chemistry. Early in the 1950s, DNA was first examined by X-ray diffraction analysis, and the X-ray diffraction results showed that DNA was composed of two strands of the polymer wound into a helix (Albert *et al*, 2002).

Human cells contain 46 nuclear DNA molecules. Each is composed of millions of nucleotide pairs (Eckert, 1997). DNA is a complex double-chained, molecule twisted into a helical form: the “double-helix” structure. The structure of DNA resembles a spiral ladder in which the “sides” are made of sugar-phosphate molecules and the “rungs” are formed from pairs of chemical known as bases or nucleotides. DNA consists of 4 bases: adenine (A), thymine (T), guanine (G) and cytosine (C). The pairing of the bases is specific: adenine is always paired with thymine, and cytosine is always paired with guanine. These four nucleotide bases represent the “genetic alphabet” and the sequences of base-pairs along the length of the DNA molecule comprise a biochemical vocabulary which encodes the genetic information essential to life processes (Curran, 1997).

Although genes are composed of DNA and contained in the chromosomes in the nucleus of the cell, only a small fraction of that DNA is actually used to form genes. Most of the DNA in a chromosome has no known function. The portion of such DNA may be more than 95% of the total complement in humans (Curran, 1997). Functional DNA is a transcribed genes and regions involved either in transcriptional regulation or in

maintaining chromosomal structure. Functional DNA comprises less than a sixth of the total human genome. Although the coding regions only comprise of 3%, the inclusion of non-expressed regions with known function, as well as pseudogenes, gene fragments and all intronic sequences which will bring the total to around 30%. The remaining 70% are extragenic DNA, comprise of repetitive DNA and unique DNA (Bennett, 2000). The repetitive DNA can be divided into two types: interspersed repetitive and tandemly repeated.

Interspersed repetitive can be classified as short and long interspersed DNA elements (SINEs and LINEs respectively). SINEs and LINEs are major contributing components in the human. Numbers of these elements may exceed a million copies in the human genome. Alu elements constitute 16.8% and 9.48% of the sequences of human chromosomes 22 and 21, respectively. There are approximately 12% of the genome is composed of Alu sequences, therefore assuming a genome size of 3 billion base pairs, between 360-390 million base pairs are Alu sequences. Since Alu elements are approximately 300 bp each, this then equates to 1.2-1.3 million copies of the repeat in the human genome (Kass, 2001).

Tandemly repeated DNA can be classified into three subgroups, known as satellite, minisatellites and microsatellites. Satellite DNA was the first tandemly repeating sequences to be discovered and was so named by its appearance as “satellite” bands that separated from the bulk DNA upon buoyant density gradient centrifugation (Bennett, 2000). Satellite DNA is a highly repetitive. It can be present in up to several million copies in a genome. The length of the repeating unit usually ranges between hundreds to thousands base pairs, and the arrays can have thousands of repetitions. These long arrays of satellite DNA form heterochromatin at and near the centromeres and telomeres

(Slamovits & Rossi, 2002). Human satellite DNA is not transcribed and there is no evidence to suggest that these restrictions upon chromosome localization are associated with any functional importance. Satellite DNA has no use for either individual DNA profiling or any genetic linkage studies due to its enormous size and restricted localization (Bennett, 2000).

Minisatellites are more interesting and useful than satellite DNA. Minisatellites DNA consist of moderate arrays of tandem repeats spanning approximately 100bp to 20kb in length. Minisatellites can be subdivided into hypervariable minisatellites DNA and telomeric DNA. Hypervariable minisatellites DNA was first discovered in 1985 by Alec Jeffrey and his colleagues at Leicester University (Bennett, 2000). Minisatellites DNA is also known as variable number tandem repeats (VNTRs). The basic repeat unit may vary in length from six to >50 nucleotides, with the overall number of repeat at any one locus usually being highly polymorphic between the individuals. This highly polymorphic feature allows it to use in the field of DNA profiling. The technique called DNA fingerprinting was developed by Jeffrey and his colleague and this involved hybridizing short synthetic probes containing core sequences, such as GGGCAGGANG to an individual's genomic DNA which had been digested with restriction enzymes (Bennett, 2000).

Another type of the minisatellites is telomeric DNA. Telomeric DNA consists of 10-15kb of hexa-nucleotide repeats (TTAGGG), added to the telomerase of all chromosomes by the enzymes telomerase. This telomeric DNA will protect the ends of chromosome from degradative enzymes or from being recognized by the cell as a broken DNA molecule in

need of repair. It also plays a role in the pairing and orientation of chromosomes during cell division (Albert *et al*, 2002).

Microsatellites or known as short tandem repeats consist of tandemly repeated 1-6bp sequences with 10-60 copies. Microsatellites are evenly distributed in all known eukaryotic genomes, and they are highly polymorphic due to variations in the number of the repeat motifs present at a particular locus within a population of individuals. The biological function of microsatellites is unknown (Hasner *et al*, 2000). Microsatellites DNA sequences are important in many types of genetic analysis, such as loss of heterozygosity testing, linkage analysis, gene mapping, paternity and forensic identity testing, evolutionary studies and clinical diagnosis (Medintz *et al*, 2001).

The microsatellites had replaced minisatellites and represent the marker of choice for many genetic applications. The replacement of minisatellites with microsatellites occurred due to the technique (VNTR probes) that applied on the minisatellites was unable to know which pairs of bands in the fingerprint represented alleles at particular minisatellites locus; consequently, it was not possible to calculate allele frequencies and hence reliably state the odds of bands within a fingerprint matching as a result of chance. Although the problems that faced at above was solved by using the multilocus minisatellites probes of DNA fingerprinting to replace the specific single locus minisatellites or VNTR probes, but most of potential for human error lay within a process known as “binning”. The “binning” was where a decision had to be made as to which allele corresponded to which band on the southern blot autoradiograph and this binning problem can be overcome by using microsatellites (Bennett, 2000).

The microsatellites or STRs have become popular in forensic laboratories because of low amounts of DNA used, even in degraded form, can be successfully typed. Sample mixtures can be more readily resolved with STR results than with previously used DNA typing technologies. In the United States, a core set of 13 STR markers are being used to generate a nationwide DNA database called the FBI Combined DNA Index System (CODIS). The CODIS Database has been successfully linking DNA profiles from repeat offenders and crime scene evidence. The STR typing also used to aid hundreds of thousands of paternity testing cases each year (Ruitberg *et al*, 2001).

STR markers for forensic use are selected taking into consideration of highly variability of discrete size of the amplified products; exhibiting low microvariants and low stutter. (Schumm, 1996). There were 12 autosomal STR loci such as CSF1PO, TPOX, TH01, vWA, D16S539, D7S820, D13S317, D5S818, F13A01, FESFPS, F13B, and LPL were selected and studied then eight of them were chosen to be validated together and combined into a commercial STR multiplex system under the name PowerPlex. The commercial STR multiplex system is normally used for population studies. There are another two STR systems, second-generation multiplex (SGM) and PowerPlex^{®/TM} 1 have been validated for paternity use. The Profiler Plus^{®/TM} STR typing system has been successfully used in ancient DNA profiling (Brettell *et al*, 2001).

OBJECTIVE OF THE STUDY

The objectives of the study are to:

1. Develop a population database of 3 validated STRs – CSF1PO, TPOX and TH01 for the Iban population in Malaysia.
2. Develop a database for individual identification use in Forensic Science institutions in the world.

MATERIALS AND METHODS

Buccal Swab from 100 Random Iban Individuals



DNA Extraction



Quantification of DNA



PCR Amplification



PAGE (Polyacrylamide Gel Electrophoresis)



Silver Stain

Figure 1: Schematic representation of analytical protocol

SAMPLE COLLECTION

125 buccal samples had been collected from unrelated Iban individuals. Before collecting the buccal sample, subjects must be fully understood about the objectives of the study and asked for the consent before started collecting the buccal swab. 1 cotton swab with both sides was used to collect buccal cells from the subject by rubbing against the inner side of subject's cheek. Collection could be done by subject himself or done by the collector with gloves. After the collection, the buccal swab must be dried in room temperature, well ventilated around 1-2 hours to make sure no moisture present, for avoiding the growth of fungus. After drying, the buccal swab must seal in the plastic envelope properly.

DEIONISED WATER

Deionised water (ddH₂O) was used in the preparation of buffer and other reagents.

CHEMICALS AND REAGENTS

Table 1: Chemicals and reagents used in the study

| No | Chemicals and Reagents |
|----|--|
| 1 | Digestion buffer |
| 2 | Protease K (20 mg/ml) |
| 3 | Phenol |
| 4 | Chloroform Isoamyl |
| 5 | Chilled Ethanol |
| 6 | 2M Sodium Acetate |
| 7 | 70% Ethanol |
| 8 | TE buffer |
| 9 | 1X TAE buffer |
| 10 | Agarose |
| 11 | 5X loading solution |
| 12 | 10 mg/ml Ethidium Bromide stock solution |
| 13 | 40% Acrylamide : Bis (19:1) |
| 14 | 10X TBE buffer |
| 15 | 10% Ammonium Persulfate |
| 16 | Urea |
| 17 | Bind saline |
| 18 | 0.5% acetic acid in 95% ethanol |
| 19 | TEMED |
| 20 | Liqui-Nox® detergent |
| 21 | Fix/stop solution |
| 22 | Silver staining solution |
| 23 | Developer solution |
| 24 | Fix/stop solution |

AMPLIFICATION MATERIALS

Table 2: Amplification materials used in the study

| No | Amplification Materials |
|----|-------------------------------|
| 1 | E-Pure water |
| 2 | STR 10X Buffer |
| 3 | Multiplex 10X Primer Pair Mix |
| 4 | <i>Taq</i> DNA polymerase |

LABORATORY EQUIPMENTS

Table 3: Laboratory equipments used in the study

| No | Laboratory Equipments |
|----|---|
| 1 | 1.5 ml microcentrifuge tubes |
| 2 | Thermal cycler PTC-200 |
| 3 | Microcentrifuge |
| 4 | 0.5 ml or 0.2 ml microcentrifuge tubes |
| 5 | Aerosol-resistant tips |
| 6 | Nalgene [®] tissue culture filter (0.2 micron) |
| 7 | Polyacrylamide gel electrophoresis apparatus |
| 8 | Glass plates |
| 9 | 14 cm vinyl doublefine sharkstooth comb |
| 10 | Power supply |
| 11 | Liqui-Nox [®] detergent |
| 12 | Clamps |
| 13 | Diamond pencil |
| 14 | Wash tubs |
| 15 | Silver multigel holder |
| 16 | Orbital shaker |
| 17 | Side spacers |

REAGENT PREPARATION FOR DNA EXTRACTION

Digestion Buffer

1M Tris-HCL (1 ml) (pH 7.5), 0.5M EDTA (2 ml), 10% SDS (20 ml), and 5M NaCl (1 ml) were mixed together. The deionised water was added and brings to 100 ml. The digestion buffer must be autoclaved before used.

Protease K (20 mg/ml)

A total of 40 mg of Protease K was added to 2 ml of sterile deionised water.