Sudan University of Science and Technology

(SUST)

College of Graduate Studies

Comparison of Some Biochemical and Molecular DNA Extraction Techniques Used in Sudan Criminal Laboratory

مقارنة بعض التقنيات البيوكيميائية والجزئية لإستخلاص الحمض النووي المستخدمة في المختبر الجنائي السوداني

A thesis submitted in fulfillment of the requirement for the master degree in clinical chemistry

By:

Amal Obeid Hag Ali

B.Se Biochemistry – Alexandria University

Supervisor

Prof. Galal Moustafa Yousif

September 2017

بسم الله الرحمن الرحيم

قَالَ تَعَالَىٰ: ﴿ وَإِذْ قَتَلْتُمْ نَفْسًا فَادَّارَأْتُمْ فِيهَا ۖ وَاللَّهُ مُخْرِجٌ مَّا كُنتُمْ تَكْتُمُونَ ﴾

سورة البقرة الآية (72)

قَالَ تَعَالَىٰ: ﴿ وَجَاءُو عَلَىٰ قَمِيصِهِ بِدَمٍ كَذِبٍ ۚ قَالَ بَلْ سَوَّلَتْ لَكُمْ أَنفُسُكُمْ أَمْرًا ۖ فَصَبْرٌ جَمِيلٌ ۖ وَاللَّهُ الْمُسْتَعَانُ عَلَىٰ مَا تَصِفُونَ ﴾

سورة يوسف الآية (18)

قَالَ تَعَالَىٰ: ﴿ إِنَّا كُلَّ شَيْءٍ خَلَقْنَاهُ بِقَدَرٍ ﴿٤٩﴾ ﴾

سورة القمر الآية (49)

صدق الله العظيم

إهداء

إلى من كلله الله بالهيبة والوقار .. إلى من علمني العطاء بدون انتظار .

إلى من أحمل أسمه بكل افتخار

(والدي العزيز)

إلى معنى الحب الحنان والتفاني .. إلى بسمة الحياة وسر الوجود

إلى من كان دعائها سر نجاحي

(أمي الحبيبة)

إلى صاحب القلب الطيب والنوايا الصادقة الى سندى وقوتى وملاذى بعد الله

(زوجى العزيز)

إلى القلوب الطاهرة الرقيقة والنفوس البريئة الى رياحين حياتى

(أبنائى)

إلى من بوجودهم أكتسب قوة ومحبة لا حدود لها.. إلى من عرفت معهم معنى الحياة

(اخوتى)

إلى روح استاذى الجليل الذى علمنى حروفا من ذهب وكلمات من درر الى من صاغ لى علمه منارة تنير لى طريق العلم

(دكتور عادل محجوب)

Acknowledgment

I would like to acknowledge and extend my heartfelt gratitude to the following persons who have made the completion of this thesis possible:

First, I would like to say Alhamdulillah, for giving me the strength and health to do this project work until it was done and made all things possible.

Iam heartily thankful to my supervisor, Prof ***Galal M. Yousif****, whose encouragement, guidance and support from the initial to the final level enabled me to develop an understanding of the subject. All too forensic laboratory administrators' staff and library members for assisting in the collection of the topics for the different chapters. Iam sincerely thankful for* ***Hashemite Kingdom of Jordan*** *for giving me an opportunity to work at their labs. I would also like to thank general administration of forensic evidence staff and friends for providing me with the inputs, facilities and motivation for the work I was able to do.*

Not forgotten to my parents and family for their unconditional love and affection specially my husband ***Nabeel Motasim*** *for the help.*

Most especially to my friends. Lastly, I offer my regards and blessings to all of those who supported me in any respect during the completion of the project.

Abstract

The advantages of DNA typing and its use for human identity testing has revolutionized law enforcement investigations in recent years by allowing forensic laboratories to match suspects with minuscule amounts of biological evidence from a crime scene. Equally important is the use of DNA to exclude suspects who were not involved in a crime or to identify the rapist in sexual crimes and in paternity cases from different samples (Evidences) from different cases were collected and extracted, amplified and analyzed for the 16 loci, and their forensic applicability were evaluated. Results were obtained by using different methods for DNA extraction. For detection of fluorescently labeled amplified products, the ABI Prism® 310 Genetic Analyzer was used. STR loci to provide a rapid, sensitive, and reliable method of DNA typing for forensic identification.

Thirty five different cases from inside and outside of Khartoum state were used. Fifteen Paternity cases, child's DNA were compared to their mothers, and the alleged father to identify child's, father, eleven cases were confirmed by their father to the child (inclusion), and four cases were denied their father (exclusion). .Ten Murder cases, the suspect's DNA profile were compared to some evidences collected from the crime scene, which led to identify the criminal, and link between victim's DNA on object in suspect's possession, Ten Rape cases were compared to suspect's DNA on victim, victim's body or clothes, five cases were matched (inclusion), four were not matched (exclusion) and one was contaminated.

المستخلص

تلعب البصمة الوراثية دوراً هام و كبير في العمل الجنائي ، إذ انها أتاحت للمختبرات الجنائية بمطابقة كميات ضئيلة من الأدلة الإحيائية الموجودة في مسرح الجريمة مع المشتبه بهم .
بنفس القدر من الأهمية ، يستخدم الحمض النووي في استبعاد المشتبه فيهم ال ذين لم يثبت إشتراكهم في إرتكاب الجريمة،وإمكانية الوصول إلى الجناة في الجرائم الجنسية ، وفي قضايا النسب ،وأوردها لبيان أهميتها و دورها الفعال في هذه الدراسة ، تم تجميع العينات والأدلة واستخلاص الحمض النووي بالنسبة للستة عشر موقع جيني المعروفة والمعمول بها لدى مختبر الأدلة الجنائية بطرق مختلفة وإكثاره بواسطة(PCR)، وتحليله بواسطة (ABI Prism® 310 Genetic Analyzer) و ذلك على عدد (35) قضية مختلفة من داخل وخارج ولاية الخرطوم .وتم مقارنة نتائج فحص الحمض النووي وبمقارنة النمط الوراثي بين المشتبه بهم وبعض المعروضات و الأدلة من مسرح الجريمة . حيث تم تحديد شخصية صاحب الاثر والتعرف على المجرمين في(10) قضايا قتل تم التوصل إلى وجود علاقة بين أداة الجريمة التي تخص الجاني وبين المعروضات التي ترجع إلى المجني علي ه وذلك من خلال التطابق التام للنمط الوراثي في كلٍ . كما تم الفصل كذلك في(15) قضية اثبات نسب وذلك بالتعرف على الأبوين البيولوجيين وذلك من خلال تطابق نصف النمط الوراثي لكل من الأبوين مع الأبناء ، وتم التطابق في(11) قضية. كما تم ايضا الفصل في (10) قضايا الاغتصاب تم التوصل إلى وجود علاقة وتطابق النمط الوراثي للمشتبه بهم والمعروضات التي تخص المجني عليهم في (5) قضايا ولم يتم التطابق في (4) قضايا، وقضية نتيجتها خليط من الانماط الوراثية نتيجة لتلوث المعروض.

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Introduction

1.1. General introduction:-

DNA has very high discrimination power. No two people with the single (exception of identical twins) have identical DNA. Current forensic DNA genotyping systems allow achieving discrimination power of one in several billion, insuring that every DNA profile obtained is virtually unique.

Different types of cells have identical DNA component. DNA obtained from different sources from the same individual will have identical pattern, regardless of the biological origin. A DNA profile of seminal stain from crime scene can be compared to that of a droplet of blood recovered from a different scene and if the semen and blood were deposited by the same individual, identical DNA results will be obtained (Khodskir 2007).

DNA is stable and reliable data can be produced from very old and decayed biological samples. DNA is more robust than proteins when subjected to harsh environments and capable of withstanding both natural and man-made environmental injury. The high molecular integrity of DNA allows forensic scientists to analyzed long-buried samples as well as samples that have subjected to high temperatures and chemical treatment. Even when biological material is severely degraded DNA evidence can still be produced using modern forensic approaches (e.g. PCR) (Khodskir 2007).

DNA inherited, family members have similar DNA profiles. Using sophisticated data analysis tools it is possible to identify a culprit with reasonable confidence by analyzing DNA by close relative (Khodskir 2007).

It is possible to generate millions of exact copies of DNA by specific enzymes reactions which allow genetic information from exceedingly small a mounts of biological material to be obtained. Modern day technology is so advanced that a single hair, skin flake or small droplet of sweat left at

the crime scene is often sufficient to obtain a full DNA profile, which can be used to identify the perpetrator (Khodskir 2007).

DNA testing can provide a vast amount of other information besides the conventional DNA profile. It is possible to determine the gender of the donor sample (Khodskir 2007).

The seminal discovery, in the early 1980s by Professor Alec Jeffrey's, of a technique which enabled the use of DNA for human identification purposes has revolutionized forensic science. (Khodskir 2007).

1.2. Research problem:-

The old techniques used are not accurate, so that is not exclude suspects who were not involved in a crime.

1.3. Rationale:-

DNA for human identification purposes has revolutionized forensic science. The criminal justice system now relies heavily on DNA-based evidence. All over the world, thousands of people have been convicted of various crimes with the help of DNA evidence and hundreds of wrongfully convicted people have been exonerated. DNA analysis has become an indispensable police tool, as it allows unambiguous identification of the criminal by traces of biological material left at the crime scene and the acquittal of innocent suspects, based on DNA evidence.

1.4. Objectives:-

1.4.1. General Objective:-

Application of bimolecular techniques in forensic medicine.

1.4.2. Specific Objective:-

1. To determine the source of biological evidence collected from the crime scene.
2. To obtain specific human beings profiles using different techniques for extraction DNA material from their cells.
3. To amplifying extracted DNA by using PCR technique.
4. To identify the 16 specific loci data by using ABI Prism 310 Genetic Analyzer for separating and detecting it.
5. To comparing these profiles together and presents it as evidences to court.

2 .Literature Review

2.1. What is DNA:-

The basic unit of life is the cell, which is a miniature factory producing the raw materials, energy and waste removal capabilities necessary to sustain life. Each cell contains the same program. Within the nucleus of our cells is a chemical substance known as DNA as shown in (Figure 2.1), that contains the information code for replicating the cell and constructing the needed enzymes. DNA residing in every cell of our body (exception of red blood cells, which lack nuclei) (Butler, 2015).

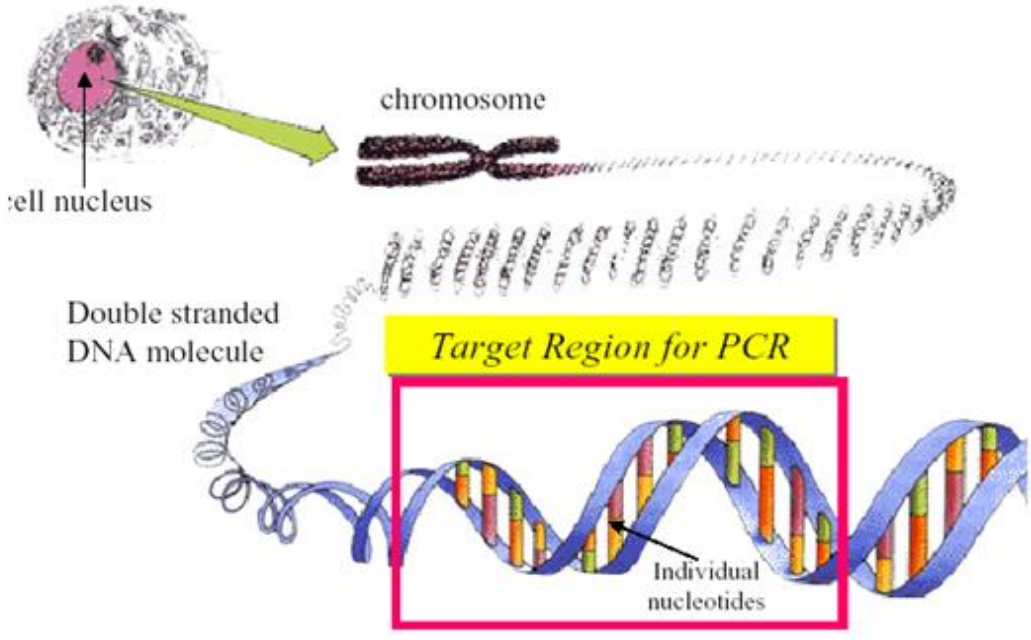


Figure 2.1: Nuclear DNA constitution (Mullis ,et al, 1993)

Deoxyribonucleic acid is nucleic acid that contains the genetic instructions used in development and functioning of all known living organisms. DNA has two primary purposes, to make copies of it so cells can

divide and carry on the same information, and to carry instruction on how to make proteins so cells can build the machinery of life. Information encoded within the DNA structure itself is passed on from generation to generation with one-half of person's DNA information coming from their mother and one-half coming from their father (Butler, 2015).

Human DNA consists of about 3 billion bases. A vast majority of our DNA molecules ((over 99.7%)) is the same between people. Only a small fraction of our DNA ((0.3% or ~10 million nucleotides)) differs between people and makes us unique individuals. These variable regions of DNA provide the capability of using DNA information for human identity purposes. Methods have been developed to locate and characterize this genetic variation of specific sites in the human genome (Butler, 2015).

2.2. Human genome:-

Human genome (complete set of genetic material), describe the total genetic information present in form of DNA resides in the nucleus (99.995%, nuclear DNA) and rest in mitochondrial (0.005%, Mitochondrial DNA). Nuclear genome provides the great bulk of essential genetic information most of which specifies the polypeptide synthesis on the cytoplasm.

The human genome had fewer genes than expected, with only about 1.5% coding for proteins, and the rest comprised by RNA genes, regulatory sequences, introns and controversially so-called junk DNA .

2. 3. Nuclear DNA:-

With human cells, DNA founds in the nucleus of the cell (nuclear DNA) is organized in structure called chromosome, which are dense packets of DNA and protection proteins called histons. The human genome is composed of 23 pairs of chromosome, each of which contains hundreds of genes separated by Intergenic region. Intergenic regions may contain regulatory sequences and non-coding DNA (International Human Genome Sequences Consortium 2004).

2. 3.1.The chromosome:-

In the nucleus of each cell, the DNA nucleus is packed into thread-like structure, called chromosome. Each chromosome is made up of DNA tightly coiled many times around proteins called histons that support its structure.

Chromosomes are not visible in the cell nucleus (not even under microscope) when the cell is not dividing. However, the DNA that makes up chromosomes becomes more lightly packed during cell division and is then visible under microscope. Most of researchers know about chromosomes was learned by observed chromosome during cell division (Kitsberg, 1991).

The human genome consists of 22 matched pairs of autosomal chromosomes and two sex-determining chromosomes. Thus, normal human cells contain 46 different chromosome (or 23 pairs). Males are designated XY because they contain a single copy of X chromosome and a single copy of Y chromosome, while Females contain two copies of X chromosomes and designated XX. Most human identity testing is performed using markers on the autosomal chromosomes, and gender determination is done with markers on the sex chromosomes(Tjio & Leven, 1956).

Chromosomes in all body cells are in diploid state; contain two sets of each chromosome. On the other hand, gametes (sperm or egg) are in a haploid state, they have only a single set of chromosome. When an egg cell and a sperm cell combine during conception, the resulting zygote becomes diploid again. Thus, chromosome in each chromosomal pair is drived from each parent at the time of conception (Butler, 2015).

Each chromosome, as shown in (Figure 2.2), has constriction point called the centromere, controls the movement of chromosome during cell division. The centromere divides the chromosome into two sections (arms). The short arm of chromosome is labeled "P arm". The long arm of chromosome is labeled "q arm",which terminate with telomeres.The location of the centromere on each chromosome gives the chromosome its

characteristic shape, and can be used to help describe the location of specific genes (Bruski et al, 1999).

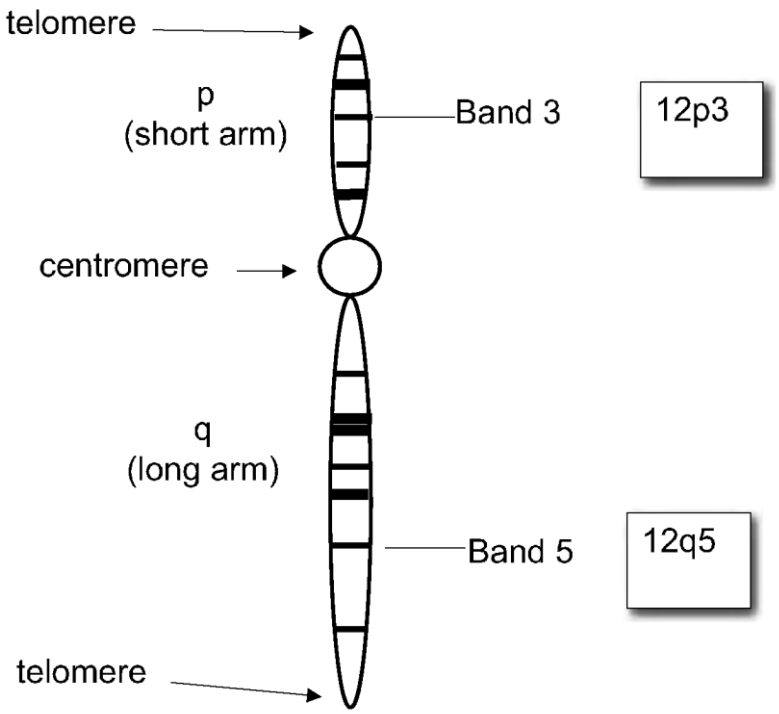


Figure 2. 2: Illustrates the shape of the chromosome (Butler, 2015)

Human chromosomes are numbered based on their overall size with chromosome I being the largest and chromosome 22 the smallest. The complete sequence of chromosome 22 was reported in December 1999 to be over 33 million nucleotides in length. Since the human genome project completed its great effort in April 2003, now know the sequence and length of all 23 pairs of human chromosomes (Butler, 2015).

During most of a cell's life cycle, the chromosome exists in an unraveled linear form. In this form, it can be transcribed to code for proteins. Regions of chromosomes that are transcriptionally active are known as euchromatin. The transcriptionally inactive portions of chromosomes, such as centromere, are heterochromatin regions and are generally not sequenced due to complex repeat patterns found therein. Prior to cell division, during the metaphase step of mitosis, chromosomes are visualized under a light

microscope as consisting of a continuous series of light and dark bands results because of different amount of A and T versus G and C based across the chromosome (Butler, 2015).

A common method for staining chromosomes to obtain a banding pattern is the use of Giemsa dye mixture that results in so-called "G-band" via G-staining method. These G-bands serves as signposts on the chromosome highway to help determine where a particular DNA sequence or gene is located compared to other DNA markers. The differences in chromosome size and banding patterns allow autosomal chromosomes and sex chromosomes to be distinguished from one another, and analysis called karotype (Butler, 2015).

DNA or genetic marker is physically mapped to a chromosome location using patterns on the metaphase chromosomes. Bands are classified according to their relative positions on the short arm "p" or the long arm "q" of specific chromosome. Thus, the chromosomal location 12p1 means band 1 on the short arm "p" of chromosome 12. The band number increase outward from the centromere to the telomere portion of the chromosome. Thus, band 3 is closer to the telomere than band 2. When a particular band is resolved further into multiple bands, its components are named P11, P12 etc. If additional sub-bands are seen as techniques are developed to improve resolution, then these are renamed P11.1, P11.11 etc. For DNA markers close to the terminal ends of the chromosome, the nomenclature "ter" is often used as a suffix to the chromosome designation. The location of a DNA marker might therefore be listed as 15qter, meaning the terminus of the long arm of chromosome15. Sometimes a DNA marker is not yet mapped with a high degree of accuracy in which case the chromosomal location would be listed as being in a particular range, i.e., 2p23-pter or somewhere between band 23 and the terminus of the short arm on chromosome 2 (Butler, 2015) .

2. 3.2. The genes:-

Each DNA molecule contains many genes, the basic physical and functional units of heredity (Oswald et al., 1944).A gene is a specific sequence of nucleotide bases, whose sequences carry the information required for constructing proteins, which provide the structural components of cells and tissues as well as enzyme for essential biochemical reactions. The human genome is estimate to comprise more than 20,000 to 25,000 genes (International Human Genome Sequencing Consortium, 2004).

Human genes vary widely in length, often extending over thousands of bases, but only about 10% of genome is known to include the protein coding sequence "axons" of genes. Interspersed within many genes are introns sequences, which have no coding function. A part of this non-coding DNA is comprised of repetitive sequences (Gilbert, 1978).

2. 4. Mitochondrial DNA:-

Mitochondria are tiny organelles that live in the cytoplasm of cells, the fluid-filled space between the cell nucleus and outer membrane. There are thousands of mitochondria in each cell, and each one has its own small double-helical circle DNA (Stryer, 1995).

Mitochondrial DNA is so special and so useful, because it's unique inheritance. Human eggs are full of mitochondria, while sperm has only a hundred or so, just enough to power it while it swims towards the egg. After fertilization, when the sperm penetrates the egg, these few male mitochondria are immediately destroyed. This mean that, while all receive our nuclear DNA (with the exception of X and Y sex chromosome) from both parents, get all of our mDNA from our mothers. She got it from her mother, who gets it from hers, and so on back in time (Wills, 1994).

mDNA changes much more rapidly than nuclear DNA (about 20 times as fast), because mitochondria lack an efficient proofreading system to check for errors when DNA is copied. The high mutation rate means that

there is plenty of variation in the sequence of mDNA between individuals, and variation is the lifeblood of genetics (Sykes, 2003).

2. 5. Structure of DNA:-

DNA is polymer of deoxyribonucleotide units. Nucleotides consist of a nitrogenous base, a sugar and phosphate group.The sugar is deoxyribose (sugar lacks an oxygen atom) that is present in ribose. The nitrogenous base is a derivative of a purine or pyrimidine, the purines in DNA are adenine (A) and guanine (G), and pyrimidines are thymine (T) and cytosine (C). A nucleoside consists of a purine or pyrimidine base bonded to sugar. In a deoxyribonucleoside, N_9 of a purine N_1 of a pyrimidine is attached to C_1 of deoxyribose. A nucleotide is a phosphate ester of a nucleoside. The most common site of esterification in naturally occurring nucleotides is the hydroxyl group attached to C_5 of the sugar(Levene, 1919)..

The backbone of DNA, which is invariant throughout the molecule, consists of deoxyriboses linked by phosphate groups. Specifically, 3'-hydroxyl of the sugar moiety of one deoxyribonucleotide is joined to the 5'-hydroxyl of adjacent sugar by phosphodiester bridge. The variable part of DNA is its sequence of four kinds of bases (A, G, C, and T) (Stryer, 1995).

The most common form of DNA in a cell is in a double helix structure (Watson and Cricks, 1953)(Figure 2. 3), in which two individual DNA strands twist around each other in a right-handed spiral. In this structure, the base pairing rules specify that guanine pairs with cytosine and adenine pairs with thymine (each pair contains one purine and one pyrimidine). The base pairing between guanine and cytosine forms three hydrogen bonds, while the base pairing between adenine and thymine forms two hydrogen bonds. The two strands in a double helix must therefore be complementary, that is, their bases must align such that the adenines of one strand are paired with the thymine’s of the other strand, and so on(Figure 2.3).

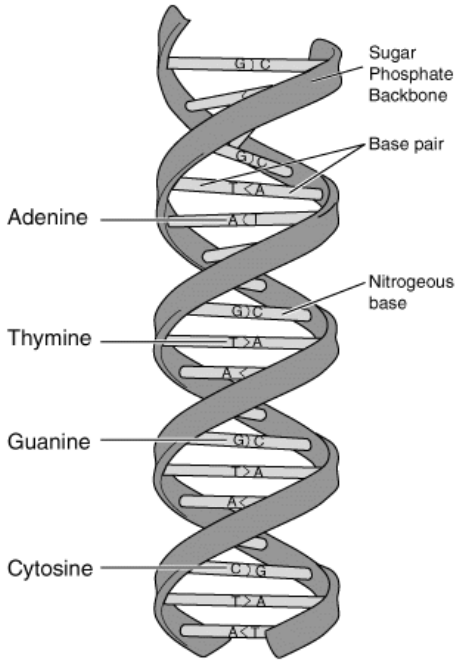


Figure 2. 3: DNA double helix structure (Watson and Cricks, 1953)

Due to the chemical composition of the pentose residues of the bases, DNA strands have directionality. One end of a DNA polymer contains an exposed hydroxyl group on the deoxyribose; this is known as the 3' end of the molecule, (Figure 2.4). The other end contains an exposed phosphate group, this end the 5′ end. The directionality of DNA is vitally important to many cellular processes, since double helices are necessarily directional (a strand running 5' → 3' pairs with a complementary strand running 3' →5') and processes such as DNA replication occur in only one direction. All nucleic acid synthesis in a cell occurs in the 5'→ 3' direction; because new monomers are, add via a dehydration reaction that uses the expose 3' hydroxyl as a nucleophile (Perulz, 1969).

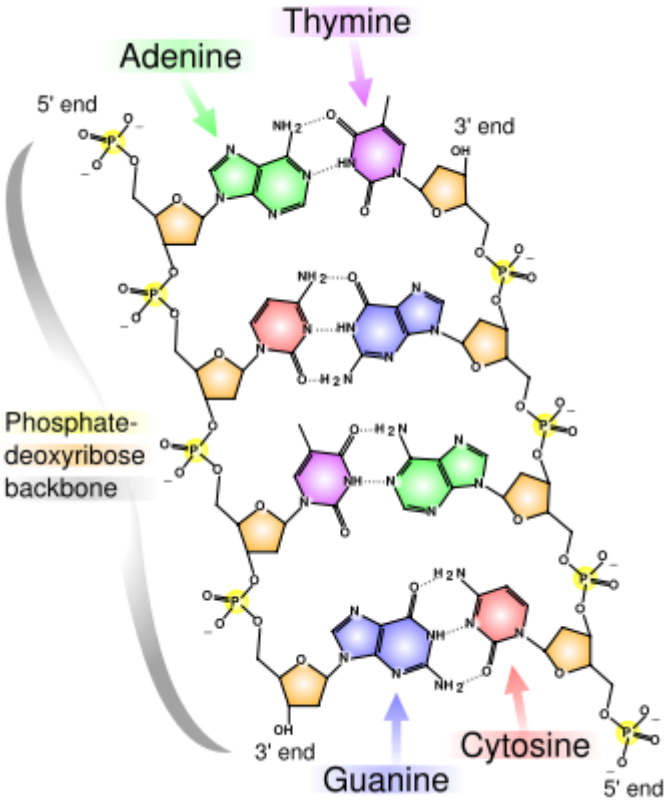


Figure 2.4: Chemical structure of DNA (Perulz,1969)

2.6. Properties of DNA:-

The weak interactions that maintain DNA in the double-stranded state are rather easily disrupted, and the two strands may thus be unwound and separated. This process, termed denaturation or melting, is an essential part of the most common DNA typing tests in use at this time. High temperature or high pH can be used to promote denaturation, although alkaline conditions are less likely to cause breakage of DNA than is elevated temperature. Low pH will also promote denaturation, but this condition must be avoided due to the tendency of DNA to release purine bases (A, G) at pH 4 or less and to hydrolyze phosphodiester bonds at pH 1(Mark et al, 1991).

In addition to extended lengths of time, hybridization requires conditions of ionic strength and temperature. The temperature must be high enough to disrupt any inter-strand base pairs, but low enough to allow the stable formation of intra-strand base pairs. The optimal temperature is 20-25º C below the melting temperature (T_m) (Mark et al., 1991).

Increasing the salt concentration stabilizes base pairing and, therefore, increases the tendency of complementary single-strands to re-nature, this effect is due primarily to shielding of the electrostatic changes of the negatively charged phosphate groups by sodium ions and by a decrease in the solubility of unpaired bases at elevated salt levels. A typical salt concentration in hybridization reactions ranges from 0.15 M to 1.0 M.

Hybridization reactions are usually performed under conditions that favor the formation of duplex DNA. Duplex that are imperfectly paired can be subsequently eliminated by high-temperature and low-salt concentration, i.e. so-called stringent conditions. Reducing the stringency of hybridization conditions encourages the maintenance of duplex containing mismatched or otherwise unpaired bases (Mark et al., 1991).

2.7. DNA Variation:-

Variations are found all throughout the genome, on every one of the 46 human chromosomes. The majority of variations are found outside of genes in the (extra) or (junk) DNA that does not affect a person's characteristics. (Craig, 2003).

Junk DNA or non-coding DNA composed of repetitive sequences, which can be divided into two classes: tandemly repetitive sequences and interspersed repeats. These tandemly repeated sequences become known as variable number of tandem repeat.

2.7.1. Variable number of tandem repeat (VNTR):-

In addition, called minisatellite, was the first DNA marker system successfully used for human identification. VNTRs are short identical segments of DNA interspersed in the human genome but clustered near the end of the chromosome. The usual length of repeat unit is 6-100 nucleotides, it repeated up to several hundred times (Khodskir 2007).

The most common method of VNTR analysis is using restriction fragment length polymorphism (RFLP) to cover genetic variation. This

method detection based on a remarkable ability of some enzyme (restriction enzyme), to recognize and cut a specific DNA sequence. If the DNA flanking a VNTR is cut with restriction enzyme, the size of the resulting DNA fragment can vary depending on repeat units within VNTR. The resultant small DNA fragments are then separated on a gel under electrical current by the process of DNA electrophoresis. The smaller the restricted fragment (i.e. the smaller number of repeated nucleotides) the faster it migrates in the gel. After the separation, the DNA fragments are transferred onto nylon membrane and made visible by a radioactivity labeled DNA probe which has homogenous sequence to the fragment of interest and under special conditions, recognizes and bind to it (Khodskir 2007).

VNTR has several disadvantages, which limited its applications. Radioactivity labeled DNA probes are usually used for detection VNTR. So special premises equipped for radioactive work are needed. It typically takes several days or even weeks to obtain DNA profile. The technique also relies on starting with a big volume of biological material and on a good quality DNA. VNTR genotyping technique is slow, cumbersome manual and could not be automated. All this prompted a shift towards PCR based DNA markers, like STR which enables automating of DNA profiling (Khodskir 2007).

2.7.2. STR analysis:-

Short tandem repeat (STR) analysis is now the most widely used DNA typing procedure as it provides higher discriminating power than RFLP analysis but it also requires a smaller sample size. Short tandem repeats (STRs) are loci on the chromosome that contain short sequence elements that repeat themselves within the DNA molecule. The repeating sequence is usually 3 - 8 bases in length (Figure 2. 5), and the entire strand of an STR is fewer than 400 bases in length. Since the strands are significantly shorter than those used in RFLP analysis(Craig, 2003).

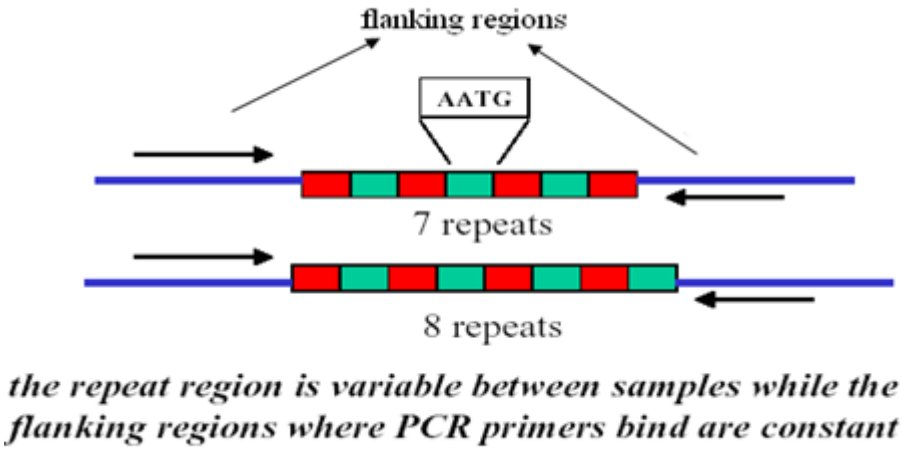


Figure 2. 5: Short tandem repeats (STRs) (Craig, 2003)

During forensic examination, an STR with a known repeat sequence is extracted and separated by electrophoresis in the same way as RFLP analysis. The effectiveness of this technique has lead to multiplexing, the extraction and analysis of a combination of different STRs. Combining the technology of PCR with STR analysis enables the simultaneous extraction and amplification of the DNA fragments. The selection of STRs ensures complete separation and clarity of results and can also include the use of fluorescent dyes to visualize the STR loci. The United States forensic science services have developed a standard set of thirteen STR loci known as the CODIS (Combined DNA Index System) (Butler, 2015).

Although gel electrophoresis is an adequate method of separation for STR loci, the speed of the extraction and amplification step and the need for sample automation has lead to the introduction of capillary electrophoresis as the preferred separation technique. The principles are similar to those used in gel electrophoresis, although the flow of matter submitted to electric potential is through a capillary column rather than an agarose well.

STR repeat sequences are named by nucleotides repeated next to other over and over again. Trinucleotides have three nucleotides in repeat unit, tetra-nucleotides have four, penta-nucleotides and hexa-nucleotides have five

and six repeat units in the core repeat respectively. Tetra nucleotides repeats have become the most popular STR markers for human identification.

The advantages of using tetra nucleotide repeat STRs include:

- A narrow allele size range that permits multiplexing.
- A narrow allele size range that reduces allelic dropout from preferential amplification of smaller alleles.
- The capability generating small PCR product sizes that benefit recovery of information from degraded DNA specimens.
- Reduced stutter product formation compared to dinucleotide repeats that benefit the interpretation of small mixture.

STRs are often divided into several categories based on the repeat pattern. Simple repeats contain unit's identical length and sequences, compound repeats comprise two or more adjacent simple repeats, and complex repeats may contain several repeat blocks of variable unit length as well as variable intervening sequence (Urquhart et al, 1994). Complex hypervariable repeat also exist with numerous non-consensus alleles that differ in both size and sequences and are therefore challenging to genotype reproducibly (Urquhart et al 1993; Gill et al 1994). This last category of STR markers is not as commonly used in forensic DNA typing due to difficulties with allele nomenclature and measurement variability between laboratories. Not all alleles for a STR locus contain complete repeat units. Even simple repeats can contain non-consensus alleles that fall in between alleles with full repeat units. Microvariants are alleles that contain incomplete repeat units. The most common example of a microvariant is the allele 9.3 at the THOI locus, which contain nine tetra nucleotide repeats and one incomplete repeat of three nucleotides because the seventh repeat is missing a single adenine out of the normal AATG repeat unit (Puers et al 1993).

2.8. DNA Markers:-

Human identity tests focus on STR markers. STR markers are genetic loci consisting of repeated subunits, 2-6 base pairs in length. Discrimination between individuals is possible because the number of subunits present for a given marker varies from person to person. Simultaneous analysis of several STR markers allows for the compilation of a profile, which is almost to a given individual (Butler 2015).

STR markers suitable for forensic analysis occur on both autosomal (non-sex) chromosome 1-22 and the sex chromosome (X and Y). Research on the use of STR markers for identification purposes has focused on autosomal markers. The most obvious reason for this is the fact they can be used for the identification of individuals from both sexes. They also have an excellent capacity to discriminate between individuals. Despite their advantages autosomal markers have significant limitation when used to analyze mixed male/female stain (Butler 2015).

STR becomes popular DNA repeat markers because they are easily amplified by the polymerase chain reaction without the problems of differential amplification. This is due to the fact that both alleles from heterozygous individuals are similar in size since the repeat size is small. The number of repeat in STR markers can be highly variable among individuals, which make these STRs effective for human identification purposes (Butler 2015).

2.8.1. Nomenclature for DNA markers:-

The nomenclature for DNA markers is straightforward. If a marker is a part of a gene or falls within a gene, the gene name is used in the designation. For example, the short tandem repeat (STR) marker TH0I is from the human tyrosine hydroxylase gene located on chromosome 2. The "01" portion of TH0I comes from the fact that the repeat region in question is located within intron I of the tyrosine hydroxylase gene. Sometimes the prefix HUM- is

included at the beginning of the locus name to indicate that is from the human genome (Butler 2015).

DNA markers that fall outside of gene regions may be designated by their chromosomal position. The STR loci D5S818 and DYS19 are examples of markers that are not found within gene regions. In these cases, the 'd' stands for DNA. The next character refers to chromosome number 5 for chromosome 5 and Y for the Y chromosome. The 'S' refers to the fact that the DNA marker is a single copy sequences. The final number indicates the order in which the marker was discovered and categorized for a particular chromosome. Sequential numbers are used to give uniqueness to each identified DNA marker (Butler 2015).

2.8.2. National DNA databank:-

The federal bureau of investigation (FBI) of US has been a leader in developing DNA typing technology for use in identification of perpetrators of violent crime. In 1997, the FBI announces the section of 13 STR loci to constitute the core of the United State national database, CODIS. All CODIS STR are teterameric repeat sequence. All forensic laboratories that use the CODIS system can contribute to a national database.

There are many advantages to the CODIS STR systems:

- The CODIS system has been widely adopted by forensic DNA analysis.
- STR alleles can be rapidly determined using commercially available kits.
- STR alleles are discrete, and behave according to known principles of population genetics
- The data are digital, and therefore ideally suited for computer databases.
- Laboratories worldwide are contributing to the analysis of STR allele frequency in different human populations.
- STR profiles can be determined with very small amounts of DNA.

CODIS links DNA evidence obtained from crimes, thereby identifying serial criminals. CODIS also compares crime scene evidence to DNA profiles from offenders, thereby providing investigators with the identity of the putative perpetrator. In addition, CODIS contains profiles from missing persons, unidentified human remains and relative of missing persons. There are three levels of CODIS.

- The local DNA index system (LDIS), used by individual laboratories.
- The state DNA index system (SDIS), used at the state level to serve as a state's DNA data base containing DNA profiles from LDIS laboratories
- The national DNA index system (NDIS), managed by the FBI as the nation's DNA database containing all DNA profile uploaded by participating states (Butler 2015).

2.8.3. The 13 codis STR loci:-

Each of the 13 core STR loci has unique characteristics Appendix (Table:1), either in terms of the numbers of alleles present, the type of repeat sequence, or the kinds of microvariants that have been observed (Butler 2015).

2.8.4. Amelogenin:-

It is a single copy gene, homologues of which are located on Xp22.1-Xp22.3 and Yp11.2. This gene for Amelogenin can be used in sex determination of samples from unknown human origin through the Polymerase Chain Reaction (PCR), this commonly performed in conjunction with STR typing kit using PCR products generated from the Amelogenin gene that occurs on both the X- and Y-chromosome. A commonly used PCR primer set first published by Sullivan et al. 1993 targets a 6 bp deletion that occurs on the X-chromosome, which enables amplicons generated from the X- and Y-chromosome to be distinguished from one another when analysis

is performed to separate STR alleles. The X chromosome gene, AMELX, gives rise to a 106 bp amplification product (amplicon) and the Y chromosome gene, AMELY, a 112 bp amplicon. Hence, the AMELX contains a 6 bp deletion in the intron 1. Therefore, when the amplicons are run on an agarose gel or analyzed using ABI Prism 310 analyzer, samples from male sources (XY) show two bands or two peaks (one for the 106 bp fragment and one for the 112 bp fragment), while females (XX) show only one band or only one peak respectively. Thus, this process allows for sex determination of unknown samples (Butler 2015).

2.9. Biological evidence at crime scene:-

The different types of biological evidence can be used to associate an individual for involvement with crime. In particular, the direct transfer of DNA from one individual to another individual or to an object can be used to link a suspect to a crime scene (Lee et al., 1991) Transfer DNA could involve:

1- Suspect's DNA on victim's body or clothing.
2- Suspect's DNA at crime scene.
3- Suspect's DNA on object involve in crime.
4- Victim's DNA on suspect's body or clothing.
5- Victim's DNA on object in suspect's possession.
6- Victim's DNA at suspect's residence "car".
7- Witness's DNA on victim or suspect.
8- Witness's DNA at crime scene.

Many different types of physical evidence are commonly submitted to Forensic Science Laboratories for examinations. Initially evidence that was suitable for DNA analysis was limited to biological substances that contain nucleated cells. Common biological specimen from which DNA has been successfully isolated and typed are as follows:

- Blood and blood stain.

- Semen and semen stain.
- Saliva, bones, skin cells, hair, tissue, teeth and in addition, other biological fluids.

The quantity of DNA that can be extracted from these common biological sources will vary (Table 2. 1).

Table 2.1: Variation in the DNA quantity from common biological sources

Type of samples	Amount of DNA
Liquid blood	20,000-40,000 ng/ml
Blood stain	250-500 ng/cm²
Liquid semen	150,000-300,000 ng/ml
Postcoital vaginal swab	10-3,000 ng/swab
Hair (with root)	1-750 ng/root
Liquid salvia	1,000-10,000 ng/ml
Oral swab	100-1500 ng/swab
Urine	1-20ng/ml
Bone	3-10 ng/mg
Tissue	50-500 ng/mg

Note that, in practice, crime scenes sample may contain considerably less usable DNA depending on environmental conditions.

2.9.1. Documentation of DNA evidence:-

The location and condition of any biological evidence must be thoroughly documented before its collection.Careful evidence documentation at the crime scene, autopsy room and Forensic Laboratory is essential. In criminal investigation, documentations havem great bearing on whether the evidence can later be introduced in court. Evidence should not be processed or moved until its original condition documented.

2.9.2. Collection and preservation of biological evidence:-

The ability to introduce DNA findings in courts is also greatly impacted by evidence collection and preservation methods. Evidence integrity, both scientific and legal, begins with first investigator at the crime scene. The specific collection method employed with depends on the state and condition of biological evidence. In general, a significant quantity of material should be collected to ensure the recovery on sufficient DNA for testing purpose. Each biological specimen should be packaged according to established Forensic practice. Once the samples have been collected, they should be promptly delivered to the forensic lab (Henry and Carll , 1998).

2.9.2.1. Collection and preservation of blood evidence:-

Most items of evidence will be collected in clean, unused paper containers such as packets, envelopes and bags. Moist or wet biological evidence (blood, body fluids) from a crime scene can be collected in clean, unused plastic containers at the scene and transported back to an evidence receiving area if the storage time in sealed plastic is less than two hours and this is done to prevent contamination of other evidence. Once in secure location, wet evidence, whether packaged in a plastic or paper must be removed and allowed to completely air dry. That evidence can then be repackaged in a new, clean, unused, dry paper container. Moisture allows the growth of microorganisms that can destroy or alter evidence.

Any items that may cross contaminate each other must be packaged separately. The containers should be closed and secured to prevent the mixture of evidence during transportations. Each container should have the collecting person's initials: the date and time it was collected; a complete description of the evidence and where it was found; and the investigating agency's name and file number.

2.9.2.2. Sample collection supplies:-

Select the appropriate sample collection box based on the type of sample to be collected.

Table 2.2: Sample collection supplies based on the type of sample

Sample type	Collection box	Short-term storage
Blood	Blood buffer "EDTA tube"	Room temperature
Blood stain	Filter paper in envelopes or FTA card	Dry, room temperature
Bone	Envelops or dry micro tubes	Dry, room temperature
Hair	Envelops or dry micro tubes	Dry, room temperature
Muscle	Tissue preservation buffer	Room temperature

2.9.2.3. Swab method:-

Some types of evidence may require that the biological sample be removed from the surrounding material before storage. This would be done to minimize contact of biological sample with potentially harmful material. For example, leather substrates may contain compounds that tend to destroy biological sample and inhibit PCR reaction, also or material not movable, in these cases, it may be wise to remove a blood stain or another stain (semen stain, salvia ….etc) by swabbing the stain as soon as possible. This process is simple, and the following outlines the procedure:

- Slightly moisten a cotton tip swab with clean water "diluted normal saline solution is as possible".

- Concentrate the stain as much as possible.
- Avoid potential sample – to sample contamination during process.
- Avoid contamination collector "wear protective clothing, change the gloves between samples"
- If cotton balls are chosen as the collection medium, Forceps used "if applicable", need to be cleaned thoroughly after each specimen.
- Air dry, never use a hair drier, and not exposure to direct sunlight.
- Package separately in paper bag or envelope.

2.9.2.4. FTA card:-

FTA cards are used for collecting buccal cells, blood samples and solid tissues. Each card is pre-assembled with all components necessary for the sample collection and long-term storage.

2.9.2.4.1. Features of the FTA card:-

- Designed to kill or rapidly inactive organisms including blood bone pathogens and prevent the growth of bacteria and other microorganisms
- Archive samples at room temperature in a dry environment .
- Reduces potential for cross-contamination between sample.
- Simply apply the sample to the FTA card and store for long time or send to forensic laboratory .
- Samples collected on FTA cards are so safe they can be shipped via regular us postal service without hazardous labeling.
- Unused cards are stored in original packaging in a cool, dry, clean environment. (Burgoyne et al., 1994).

2.9.3. Storage of biological material:-

Biological material collected for DNA analysis should be stored in conditions that will slow the rate of DNA degradation, in particular low temperatures and low humidity. A cool and dry environment limits the action

of bacteria and fungi that find biological material a rich source of food and can rapidly degrade biological material.

The exact conditions depend on the nature of the samples and the environment in which the samples are to be stored. Buccal swabs and swabs used to collect material at a crime scene can be stored under refrigeration for short periods and are either frozen directly or dried and then stored at -20°C for longer term storage. Blood samples will normally be stored at between -20°C and -70°C. Buccal and blood samples collected using FTA cards can be stored for years at room temperature. Some items of evidence, like clothing, can be stored in a cool dry room, in temperate regions of the world DNA has been recovered from material stored at room temperature for several years. When samples are not frozen, for example clothing, they are stored in acid-free paper rather than plastic bags, to minimize the building up of any moistures. Once the DNA has been extracted from sample, the DNA can be stored short term at 4°C but should be stored at -20°C to -70°C for long-term storage (Benecke, 2005) .

2.9.4. Reference samples:-

In order to identify samples recovered from the crime scene, reference samples are needed for comparison. Reference samples are provided by suspect and, in some cases, a victim. Traditionally, blood samples have been taken and these provide an abundant supply of DNA: however, they are invasive and blood samples are potential health hazard. Buccal swabs that are rubbed on the inner surface of the cheek to collect cellular material have replaced blood samples in many scenarios. In some circumstances plucked hairs may be used, this source of material is not commonly used.

2.10. Methods for measuring DNA variation:-

There are some techniques used by forensic DNA laboratories for human identity (Rudin, 2002).

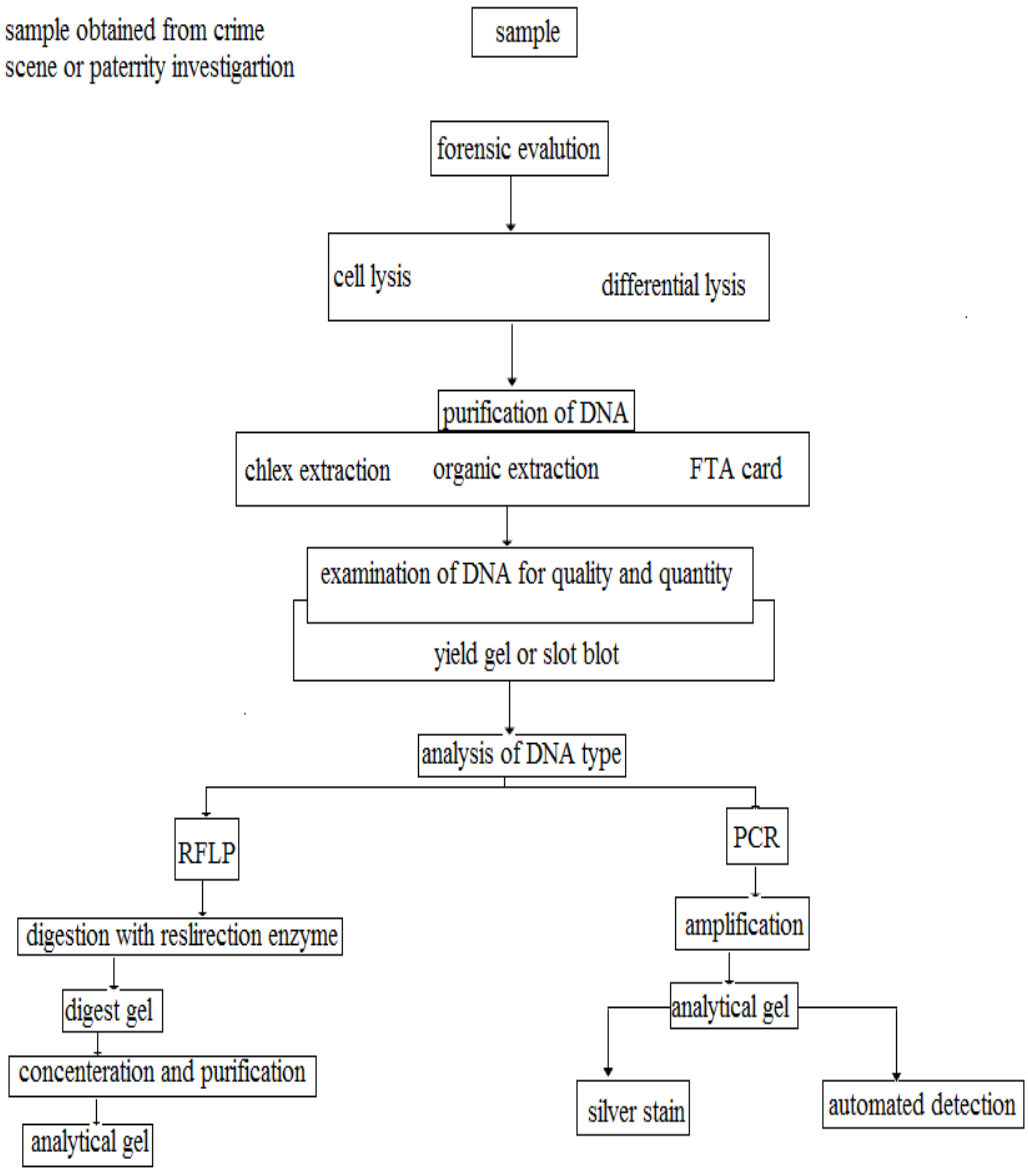


Figure2. 6: Steps of measuring DNA variation (Rudin, 2002).

Note: recently Maxwell 16 Instrument is used for purification of DNA

2.10.1. Restriction fragment length polymorphism (RFLP Method):-

Is a technique where the genomic DNA is treated with one or more restriction enzymes which cut the DNA at specific four or six base pair sequences called restriction site.

RFLP typing of purified DNA consist of the following steps:

1. Cutting the DNA in to pieces (Restriction enzyme digestion).
2. Separating the DNA by size (Gel Electrophoresis).
3. Transferring the DNA to a solid supported surface (southern transfer).
4. Targeting and visualizing the DNA of interest (Hybridization and Autoradiography) and then reading the DNA profile.

2.10.1.1. Restriction enzymes:-

Hundreds of different restriction enzymes have been isolated from bacteria. Each one cuts DNA at a specific base sequences. For example, EcoR1 always cuts DNA at GAATTC as indicated below:

The sequence GAATTG appears three times in the DNA strand below. As a result, the strand is cut in to four pieces.

GTAAGAATTCTTTAGAATTCCGCCATTATCGAATTCAGGATCTTAC
CATTCTTAAGAAATCTTAAGGCGGTAATAGCTTAAGTCCTAGAATG

GTAAG AATTCTTTAG AATTCCGCCATTATCG AATTCAGGATCTTAC
CATTCTTAA GAAATCTTAA GGCGGTAATAGCTTAA GTCCTAGAATG

Other restriction enzymes cut at different sites, some examples are listed below.

Enzyme	cutting site
Bam HI	GGATCC
Hae III	GGCC
PST I	CTGCAG
Hinf I	GANTC

In RFLP analysis, the DNA is cut into fragments. A large number of short fragments will be produced. Restriction enzymes always cut at the same base, because no two individuals have identical DNA, no two individuals will have the same length fragments. For example, the enzyme EcoRI always cuts DNA at the sequences GAATTC. Different people are going to have different numbers of this particular sequence and will therefore have different fragment length. In addition, some of them will be at different location on the chromosome (Michael,1994).

2.10.2. Gel electrophoresis:-

Electrophoresis is a technique used to separate the DNA fragments according to their size. They are placed on a sheet of gelatin and an electric current is applied to the sheet. DNA is charged and it move into an electric field toward the positive pole.

In Figure 2.7, holes (wells) in the gelatin can be seen. DNA samples placed in these wells will migrate through the gelatin toward the positive side after an electric current is applied.

The smallest fragments will move the fastest, they are able to move through the pores in the gelatin faster. Bands will be produced on the gelatin where the fragments accumulate. The shortest fragment accumulate near one end of the gelatin and the longer, slower moving ones remain near the other end. The DNA can then be rendered visible by variably of methods, it

can be stained and see under ultra violet light, or by doing a southern blot (Bordy, 2004).

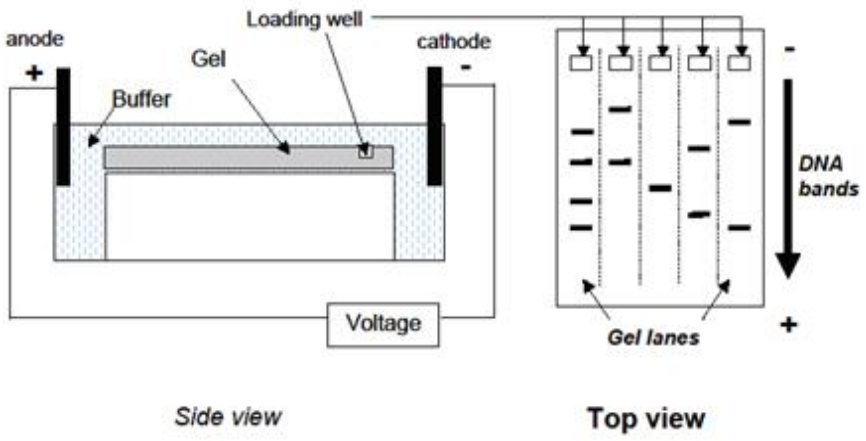


Figure 2.7: Diagram of gel electrophoresis

2.11. Problems with DNA typing:-

A DNA "print" is composed of a set of bands on an autoradiogram to determine whether two samples could have a common source; an analyst must compare their DNA prints. Whether the analyst declares the prints a "match" (inclusion), not a match (exclusion), or inconclusive depends on how closely the bands of one print appear to align with corresponding bands in the others (i.e. whether corresponding bands appear to have the same molecular weight). Consequently, the position of the bands within a DNA print, relative to other bands and relative to the position of reference bands known as molecular weight markers, is of paramount importance in determining identity. Any factor that is likely to affect the position or numbers of bands in a DNA print will affect the confidence with which a determination of identity can be made. There are some factors that can alter the appearance of DNA prints, grouping into three categories: those that caused position of the bands to shift up or down relative to standard markers and other prints, those that cause the appearance of extra bands, and those that cause the disappearance of bands (Allelic dropout) (Garner, Revzin 1981).

2.11. 1. Band shift:-

In forensic RFLP analysis, bands from the same source but in different wells on a gel are sometimes misaligned, the banding batterns may be displaced up or down relative to each other or relative to marker DNA pattern, this phenomenon causes due to variety of factors; including:

Over loading of gel wells with excessive amounts of DNA. In experimental studies, overloading has indeed been shown to cause band shift, although the shifts observed in the experiments are downward (Johnson et al., 1980). Band shift is sometimes observed on autorads that have marked lane-to-lane variation in the intensity of band, there are two types of control procedures that can be used to minimize lane-to-lane

overloading. One approach currently used by all forensic DNA laboratories, is simply to quantify the DNA in each sample before discrepancies in band intensity and to reduce the likelihood of electrophoresis to assure that roughly equal amounts are added to each gel well. Spectroscopy in widely accepted as the preferred for quantifying DNA.). In the absence of reliable methods for quantization, use serial dilution, to assure accurate comparison of unknown DNA samples to standard, a number of standards of DNA samples are applied in a dilution range. To date this method has not been adopted by forensic DNA laboratories(Maniatis et al., 1982.

Band shift may also caused by the presence of contaminating materials in forensic samples, such as soil, clothing dyes, foreign proteins that survive the DNA extraction and purification process or the introduction of contaminating materials during the extraction process (manual extraction). Forensic samples may contain a number of types of molecules known to bind to DNA. It is well established that proteins will bind to DNA under some conditions and, as result, retard the mobility of DNA during electrophoresis (Crother 1987and Revzin 1989). Indeed, scientists often rely on this phenomenon to study the interaction of protein and DNA, identifying DNA sequences at which a protein might bind by looking for a characteristic (gel retardation) of restriction fragment (Garner, Revzin 1981). Although protocols for forensic DNA testing typically include an enzymatic digestion stage to remove proteins, and the kind of binding that proteins exhibited can be dissociated by heat treatment of DNA sample before loading it on the gel, under some circumstances proteins may escape digestion or become irreversible bound to DNA (for example, cross-linking may occur due to exposure to ultraviolet light) such that heat denaturation would be ineffective (Smith et al., 1980).

Dyes may also interact with DNA in a manner that can cause band shift; it is known that some dyes interact with the DNA molecule by a

process known as “intercalation”. There have also been reports that some clothing dyes cause band shift.

Humic acid, which is found in soil, may also contribute to band shift and poses special problems, because it is similar in structure and properties to DNA. As such it co-purifies with DNA. The mechanism by which humic acid leads to band shift is not known, one possibility is that it contributes to the overloading problem by interfering with accurate quantization of DNA (humic materials absorb light in a range similar to that absorb by DNA, their presence may interfere with accurate quantitation of DNA by optical densitometry. After a recent study on the effects of contaminations on band shift, cellmark concluded that “with possible exception of the detergent SDS, the components used normally in the extraction of DNA from stains presented as evidence are not likely to cause the type of band shifting observed in casework”. A possible method for distinguishing band shift from true genetic difference in the DNA samples being tested is the use of a “constant band” or monomorphic probe. Such a probe would always identify DNA fragments of a known length and thus would produce a band at a known position. This “constant band” would serve as benchmark for determining the direction and degree of shift in each track (well) (Garner, Revzin 1981).

2.11.2. Extra band:-

When comparing two DNA prints (A & B) analysts sometimes find all of the bands in print A align with the corresponding band in print B. But the print B also contains “extra bands” not appear in A. Extra bands create ambiguity in the interpretation of DNA prints because they have a number of possible explanation. The simplest explanation is that there is a genetic difference between the two samples (i.e. the samples do not match). However, extra bands may also appear for a variety of other reasons, including mixing of sample at the crime scene, cross-contamination

of samples (at the crime scene, or in the laboratory), problem in restriction digestion, problems in hybridization, or the presence non-human DNA (e.g. from bacteria) (Garner, Revzin 1981).

2.11.3. Sample cross-contamination:-

Cross-contamination of samples is a constant danger in molecular biology. DNA from one sample may in advertently be mixed with another sample at a number of stages (when the samples are collected, during DNA extraction procedure, when enzymes and buffers are added to samples for restriction digestion, or when samples are placed in well of the gel, and may also result from lateral movement of DNA across the gel "from one lane to adjacent lane"). Lateral movement may occur during electrophoresis due to defects in the gel (rarely), during southern blotting (as result of capillary action when the nylon membrane is placed in contact with the gel or remove by dragging it across the gel), or during drying "if the membrane is placed on a plotter contaminated with DNA".

Samples to be compared should never be run in adjacent lanes. A block control lane should always exist between two samples to be compared so that the spill-over and lateral movement of DNA is likely to be detected(Garner, Revzin 1981).

2.11.4. Mixed stain:-

Mixed stains are common in forensic casework. In the rape, it is normal for vaginal swabs to contain DNA from the semen of rapist and from the epithelial cells of the victim. In addition the swabs may contain semen from other sexual partners of the victim. The DNA extraction procedures used by forensic DNA laboratories take advantage of unique biological properties of the sperm cell to separate male and female DNA in such samples. It is therefore possible to fractionate a vaginal swab sample to produce a sperm DNA print of the rapist and non-sperm DNA print of the

victim. However, the separation is not always perfect; the sperm DNA print may show extra bands from the victim and the non-sperm DNA print may show extra bands from sperm. In the case of mixed blood stains, the DNA of the contributors cannot be separated.

Where a mixed stain is suspected, the laboratory should, wherever possible, run samples from all individuals who's DNA might be part of the "mix" as controls. In rape cases, a sample from the victim should always be run as control(Garner, Revzin 1981).

2.11.5. Altered enzyme specificity:-

The numbers of bands in a DNA print and their position depends on the way in which the DNA in the sample is cut by restriction enzyme. The restriction enzymes sometimes cut at the wrong sites, due to an alteration in their specificity, thereby changing the position and/or the number of bands in the resulting DNA print. Alteration in enzyme specificity fall into two categories: where the alteration results in incomplete cleavage of the DNA "partial digestion", where the alteration produces cuts at too many sites "star activity". Alteration in specificity may arise from a variety of causes; the most important factors affecting the performance of restriction enzymes are (a) the purity and physical characteristics of the substrate DNA, (b) the reagents used in the reaction, (c) the assay volume and associated errors, and (d) the time and temperature of incubation (Fuchs & Blakesley, 1989).

2.11.6. Missing bands:-

One or more bands in a DNA print sometimes fail to appear in another print of the same individuals. The most common cause of band loss is degradation of the DNA in a sample. As degradation a progress, high molecular weight DNA fragments are lost first; hence bands at the top of the gel are the first to disappear. The degraded DNA from the lost

fragment sometimes appears as background in the print, but often is not detectable.

Forensic DNA laboratories typically checked for degradation only by the use of ethiduim bromide staining of gels. This is an unreliable method, because samples are sometimes contaminated with non-human DNA, which cannot be distinguished from human DNA with this technique. Hence, contamination may hide degradation. Determining the degree of degradation and whether it is sufficient to cause the disappearance of high molecular weight bands. A better approach would be the use of high molecular weight monomorphic probe. If the probe produced a band high on the gel, the analyst would know that sufficient high molecular weight DNA was present to produce bands and would know not to attribute a missing band to degradation. If the probe did not produce a band in the expected place, it would support the conclusion that degradation had occurred (Mark and Harington, 1991).

2.12. DNA samples processing:-

The basic steps in the production of an STR profile from a biological sample are:

1. Extraction of DNA.
2. Amplification via PCR.
3. Separation of PCR products according to size of fragments .
4. Detection via staining or florescent dyes.

2.12.1. DNA Extraction and quantification:-

Biological sample obtained from crime scene contains a number of substances beside DNA. DNA molecules must be separated from other cellular material before they can be extracted. Cellular proteins that package and protect DNA in the environment of the cell can inhibit the ability to analyze the DNA therefore; DNA extraction methods have been

developed to separate proteins and other cellular materials from the DNA molecules.

The isolation of genomic DNA is crucial step in the process of DNA profiling. The success of all subsequent genotyping procedures depends on the availability of sufficient amounts of highly purified DNA from biological crime stain as well as from reference blood samples. High molecular weight DNA is usually only required for DNA profiling protocols based on southern blot analysis and hybridization with multi and single locus variable number of tandem repeat (VNTR) probes in cases in which stain samples contain at least microgram amounts of DNA. The DNA extraction yield from biological stains is difficult to estimate in advance and depends on number of unpredictable factors regardful the stain sample e.g. storage condition, exposure to heat, sunlight, moisture or bacterial and fungal contamination, all of which influence the quantity and quality of extracted DNA. (Gross-bellard 1973).

DNA extraction has two main aims : firstly to be very efficient, extracting enough DNA from a sample to perform the DNA profiling, this is increasingly important as the sample size diminished, and secondly, to extract DNA that is pure enough for subsequent analysis, the level of difficulty here depends very much on the nature of the sample. Once the DNA has been extracted, quantifying the DNA accurately is important for subsequent analysis.

There are many methods available for extracting DNA. The choice of method to use depends on a number of factors, including the sample type and quantity. The speed and in some cases ability to automate the extraction procedure, the success rate of forensic sample is a result of extracting the maximum amount of DNA from a sample and at the same time removing any PCR inhibitors that will prevent successful profiling, the chemicals that are used in the extract, most laboratories go to great

lengths to avoid using hazardous chemicals, and the cost of the procedure beside the experience of laboratory staff.

2.12.1.1: General principles of DNA extraction: -

The three stages of DNA extraction can be classified as:

- Disruption of the cellular membranes, resulting in cell lysis
- Protein denaturating
- Separating of DNA from the denatured protein and other cellular components

Some of the extracting methods commonly used in forensic laboratories are described below:

2.12.1.2. Phenol – chloroform – based (organic Method):-

Organic extraction is the mechanical or chemical disruption of cells to release their organelles and contents. This works well in samples containing many cells, but has required adaptation for use with the much smaller biological samples collected at crime scenes. A conventional method uses organic chemicals to isolate genomic DNA.

These methods are often preferred for the extraction of biological stains containing small amounts of DNA or degraded DNA. These methods could be considered less harsh than other methods, such as the use of Chelex beads, because no boiling step is required. The procedure can be described in four steps

Step One: Solubilization of Stain Components

Water must be replaced so that dried stains can be resolubilized for DNA extraction procedures. The DNA is protected from unnecessary degradation in this process by adding EDTA, a magnesium chelator, to the lysis buffer. EDTA prevents nucleases from degrading the DNA. Tris (a component of the buffer) interacts with the lipopolysaccharides present on

the outer cell membrane, which helps to make it permeable, this effect is enhanced with the addition of EDTA.

Step Two: Denaturation and Hydrolysis of Proteins

Cells are lysed using a detergent, Proteinase K, and dithiothreitol (DTT). Extractions must use appropriate salt concentration and pH to ensure that proteins and other contaminants are separated into the organic phase and that DNA remains in the aqueous phase.

Detergents, which are included in the stain extraction buffer, its function is to lyses cell membranes, separate histons proteins from DNA, denature

histone proteins, destroy secondary and tertiary structures of proteins and decreases their solubility in aqueous solution .

Sarkosyl, is generally used if a lyses procedure is conducted under refrigerated conditions (less than room temperature) because sodium dodecyl sulfate (SDS) precipitates out of solution at these temperatures.SDS is the detergent of choice when lyses procedures are conducted at room temperature.

Proteinase K is used to hydrolyze histone proteins and is well suited to the extraction process because, it is active over a wide pH range (4-12.5), it is active in the presence of SDS, and it is not affected by EDTA.

Proteinase K is produced by the fungus Tritirachium album Limber. It is an endolytic protease that cleaves peptide bonds at the carboxylic sides of aliphatic, aromatic, or hydrophobic amino acids. Proteinase K is classified as a serine protease. Proteinase K in the extraction buffer inactivates nucleases and aids in lysis of epithelial and white blood cells to free nuclear DNA. Nuclease inactivation is a very important step in DNA isolation. Nucleases naturally exist in the cell to break down the nucleic acids after they serve their functions in protein manufacture, thus allowing the individual building blocks of the DNA and RNA to be recycled by the cell. Inactivating these nucleases preserves the DNA so that it can be extracted and purified.

Dithiothreitol (DTT) reduces disulfides to dithiols, allowing release of the DNA from its protective proteins and further degradation of the proteins by Proteinase K. DTT is an essential component for sperm cell lysis because the cell membrane contains a high concentration of disulfides.

Step Three: Removal of Denatured Proteins:

Denatured proteins are removed during the extraction using a phenol chloroform isoamyl alcohol (PCI) combination. Phenol denatures proteins that are subsequently hydrolyzed with Proteinase K. Due to their negative charge, DNA molecules can be separated from other cellular components. Addition of PCI to a sample promotes separation of non-polar (organic) and polar (aqueous) phases.

The phenol is not miscible with water, denatures protein, and sequesters the denatured hydrolyzed protein in the organic phase. During this process, the DNA remains in the aqueous phase in its double-stranded state.

PCI may be purchased commercially or prepared in a ratio of 25:24:1. Because the pH of phenol (approximately 7.0) would generally be too acidic for purposes of DNA extraction, the phenol is buffered by saturation with TE buffer.

Phenols are not true alcohols; they are more acidic than alcohols but less so than carboxylic acids. In most reactions they behave as nucleophile. Phenols are also readily oxidized, more so than alcohols. This property is employed in DNA extraction. The addition of **hydroxyquinoline**, to the reagent gives the organic phase an orange color, making it easier to differentiate the aqueous phase containing DNA. Polysaccharides and proteins are soluble in phenol, allowing for their separation from DNA.

Chloroform ($CHCl_3$), or trichloro-methane, is a colorless liquid that is slightly water-soluble and miscible with organic solvents such as phenol. It is more dense than water or buffer (in which DNA is soluble), yet less dense than phenol. As it increases the phenol phase density, it promotes a sharp interface between the organic and aqueous layers. Chloroform also

solubilizes lipids. During the extraction procedure, cellular debris that is not totally digested can be observed at the interface.

Isoamyl Alcohol (3-methyl-1-butanol) is a primary alcohol. A liquid solvent, it is often included in genomic extraction protocols to help prevent foaming of the reagents, making it easier to detect the interface between the organic and aqueous phases. It is included in the protocol in very small concentration, compared to the other reagents (Baechel 1989).

Step Four: DNA Purification:

DNA can be recovered from the aqueous phase with an ethanol precipitation or using a centrifugal filter unit (Centricon®or Microcon®).

Centrifugal filter units are used to purify and concentrate DNA. Centrifugal filter units increase the concentration of DNA in solution by retaining the DNA while eliminating a portion of the fluid from the sample. Another benefit of the unit is the ability to secure DNA while contaminants (possibly PCR inhibitors) are washed from the sample.

Centrifugal filter units separate molecules by size through a series of washing and centrifugation steps. The Millipore Corporation produces two centrifugal filter units under the names Centricon® and Microcon® (Comey 1994).

2.12. 1.3. Differential method:-

Differential extraction methods are used to separate spermatozoa from other cell types. Spermatozoa are more difficult to lyse than other cells and conditions can be set so that all cells except spermatozoa are lysed. The supernatant containing the DNA from these cells is removed from the sperm cells, which can then be lysed separately.

The differential extraction steps are:

Optional wash step

Some laboratories have incorporated an optional wash step at the beginning of the procedure to remove cellular debris and contaminants. The sample is gently washed in a buffer and detergent and the supernatant is removed (wash fraction). This can be done under refrigerated conditions or at room temperature.

Non-sperm cell lysis

An extraction buffer containing a buffer, detergent, and Proteinase K is added to the sample and incubated. This step lyses all cells except spermatozoa. The supernatant containing the DNA from the lysed cells (fraction 1) is removed after pelleting the spermatozoa. The sperm pellet is often washed numerous times with a buffer to remove excess DNA from this lysis step. If this wash is not done, it is not unusual to see a low level of fraction 1 DNA in fraction 2. If any of the sperm cells are weak or otherwise compromised, these may lyse in the first step, leaving a low level of fraction 2 DNA in fraction 1.

Sperm cell lysis

The pelleted sperm cells are lysed under more stringent conditions, using a buffer, detergent, DTT, and a higher concentration of Proteinase K (fraction 2), and are subsequently incubated.

Both fractions (including the wash fraction, if appropriate) are extracted separately with the phenol/chloroform isoamyl alcohol combination and purified.

The success of differential extraction depends on the sperm head resisting the processes that readily lyse epithelial and white blood cells. the sources of DNA from different contributors to a stain, namely a male donor and female victim (Yoshida 1995).

2.12.1.4. Chelex method (inorganic):-

Procedures utilizing chelex 100 chelating resin have been developed for extracting DNA from forensic-type cells samples for use with the PCR.

The procedures are simply, rapid, involve no organic solvents and do not require multiple tube transfers for most types of samples. The extracting of DNA from semen and every bloodstains using protenase k and phenol-chloroform extracting. DNA extracted from bloodstain seems less prone to contain PCR inhibitors when prepared by this method .

Chelex 100 scavenges metal contaminates to an extremely high degree of purity without altering the concentration of nonmetallic ions.The resin is composed of styrene divinyl benzene copolymer containing paired imino diacetate ions that act as chelating groups in binding polyvalent metals ions. Chelex 100 has a particularly high selectivity for divalent ions and differs from ordinary ions exchangers because of its high selectivity for metal ions that its higher bond strength (Bio-Rad laboratories chelae 100 chelating ion exchange resin instruction manual 2004) .

The basic procedure for recovering DNA from forensic samples, such as whole blood and blood stains, consist of an initial wash step to remove possible contamination and inhibitors such as heme and other proteins, for other forensic samples, such as tissue and bone, the wash step is not necessary. The samples are the boiled in a 5% suspension of chelex 100. After a quick spin, an aliquot of the supernatant can add directly to the amplification reaction. The alkalinity of chelex 100 suspensions and exposure to 100°C temperatures result in the disruption of the cell membranes and denaturation of the DNA. The exact role of chelex 100 during the bioting process is still unclear (Walsh et al., 1991). Walsh et al shown that purified DNA that has been subjected to temperature of 100°C in distilled water alone is inactive in PCR. Therefore, the presence of the chelating resin during the boiling step may have a protective role and prevent the degradation of DNA by chelating metal ions. These ions may acts as catalysts in the breakdown of DNA in low ionic strength solutions at high temperature (Singer et al., 1989).

2.12.1.5. Extraction by Maxwell™ 16 automated DNA: - purification instrument

The Maxwell 16 System purifies samples using paramagnetic particles (PMPs), which provide a mobile solid phase that optimizes capture, washing and elution of the target DNA. The Maxwell 16 Instrument is a magnetic-particle – handling instrument that efficiently preprocesses liquid and solid samples, transports the PMP through purification reagents in the prefilled cartridges, and mixes efficiently during processing. Several Maxwell 16 reagent kits are available and allow optimal purification from a variety of sample types .

2.12.2. Polymerase chain reaction (PCR):-

The polymerase chain reaction (PCR) is a technique widely used in molecular biology. It derives its name from one of its key components, a DNA polymerase used to amplify (i.e. replicate) a small amount of DNA by invitro enzymatic replication. As PCR progresses, the DNA thus generated is used as template for replication. This sets in motion a chain reaction in which the DNA template is exponentially amplified. With PCR it is possible to amplify a single or few copies of a piece of DNA across several orders of magnitude, generating millions or more copies of the DNA peace. PCR can be performed without restrictions on the form of DNA, and it can be extensively modified to perform a wide array of genetic manipulations (Mullis et al, 1986).

2.12.2.1. Principle of PCR:-

PCR is used to amplify specific regions of a DNA strand (the DNA target). This can be a single gene, a part of a gene, or a non-coding sequence. Most PCR methods typically amplify DNA fragments of up to 10 kilo base pairs (kb), although some techniques allow for amplification of fragments up

to 40 kb in size (Cheng, 1994). A basic PCR set up requires several components and reagents. These components include:

- DNA template that contains the DNA region (target) to be amplified.
- One or more primers, which are complementary to the DNA regions at the 5′ (five prime) and 3′(three prime) ends of the DNA region.
- A DNA polymerase such as Taq polymerase or another DNA polymerase with a temperature optimum at around 70°C.
- Deoxynucleotide triphosphates (dNTPs), the building blocks from which the DNA polymerase synthesizes a new DNA strand.
- Buffer solution, providing a suitable chemical environment for optimum activity and stability of the DNA polymerase.
- Divalent cations, magnesium or manganese ions; generally Mg^{2+} is used, but Mn^{2+} can be utilized for PCR-mediated DNA mutagenesis, as higher Mn^{2+} concentration increases the error rate during DNA synthesis
- Monovalent cation potassium ions (Joseph, 2001).

2.12.2.2. PCR Primer design:-

The target region on the DNA template is defined by the position of the. Primers PCR yield is directly affected by the annealing characteristics of the primers. For the PCR to work efficiently, the two primers must be specific to the target region, prossess similar annealing temperature, not interact significantly with each other or themselves to form (primer dimer) and be structurally compatible likewise, the sequence region to which the primers bind must be fairly well conserved, because if the sequence changed from one DNA template to the next then the primer will not bind appropriately. The general guidelines to optimal PCR primer design are listed in (Table 2. 3).

Table 2.3: **General guidelines for PCR primer Design**

Parameters	Optimal values
Primer length	18- 30 bases
Primer Tm	55-72 ºC
Percentage GC content	40 -60 %
No self complementary	<3 contiguous bases
No complementary to other (primer dimer)	<3 contiguous bases specially at the 3́ end
Distance between two primers on target sequence	< 2000 bases
The difference between forward and reverse primer in pair	< 5 ºC
No long run with the same base	< 4 contiguous bases

An extraction blank is also useful to verify that the reagents used are clean of any extraneous DNA template (Presly, et al., 1994).

2.12.2.3. Controls:-

Controls are used to monitor the effectiveness of the chosen experiment conditions, the controls typically includes:

Negative control, which is the entire PCR reaction without any DNA template. The negative control usually contains water or buffer of the same volume as DNA template, and is useful to assess whether or not any of the PCR components have been contaminated by DNA.

Positive control: is a valuable indicator of whether or not any of the PCR components have not failed or were not added during the reaction. Standard DNA template of known sequence with good quality DNA should be used for the positive control. The DNA template should be amplified with the same PCR primers as used in the rest of the samples in the batch that is being amplified.

The purpose of the positive control is to ensure confidence that reaction components and thermal cycling parameters are working for amplifying specific region of the DNA.

2.12.2.4. Ampli Taq Gold DNA polymerase:-

Ampli Taq Gold TM DNA Polymerase is chemically modified enzyme that is rendered inactive until heated (Brich et al, 1996) an extended pre-incubation of 95ºC, usually for 10 minutes, is used to activate the Ampli Taq Gold. At a pH below 7.0, the chemical modification moieties fall off and the activity of the polymerase restored. The tris buffer in the PCR reaction is pH sensitive with temperature variation, higher temperatures cause the solution pH decrease by approximately 0.02 pH units with every 1ºC (Innis and Gefland, 1988) a tris puffer with the pH 8.3 at 25ºC will decrease to pH 6.9 at 95oC. Thus, not only is the template DNA well denatured but also the polymerase is activated just when it is needed, and not in a situation where primer dimer and mispriming can occur as easy.

It is important to note that Ampli Taq Gold is not copatiable with pH 9.0 buffer used for regular Ampli Taq Gold TM DNA Polymerase. This fact is because the pH does not get low enough to remove the chemical modifications on Taq Gold and thus the enzyme remains inactive. This buffer with the pH 8.0 or 8.3 at 25oC works the best with the Taq Gold (Moretti et al, 1998).

2.12.2.5. Multiplex PCR:-

The polymerase chain reaction permits more than one regions to be copied simultaneously by simply adding more than one primer set to the reaction mixture (Edwards et al., 1994). The simultaneous amplification of two or more regions of DNA is commonly known as multiplexing or multiplex PCR. For multiplex reaction to work properly the primers pair need to be compatible, in other word the primer annealing temperatures should be similar and excessive regions of the complementarily should be avoided to prevent the formation of primer – dimer. The addition of each new primer in

multiplex PCR reaction exponentially increases the complexity of possible primer interaction (Butler et al., 2004).

2.12.2.6. PCR Inhibitors:-

Generally exert their effects through direct interaction with the DNA or interference with the DNA polymerase. Direct binding of the agents to single stranded or double stranded DNA can prevent amplification. Inhibitors can also interact directly with DNA polymerase to block enzyme activity (Weyant et al., 1990).

DNA polymerase has cofactors requirements that can be the target of inhibition. Magnesium is the critical cofactor, and agent that reduced Mg^{+2} availability or interference with binding of $Mg^{+2}to$ the DNA polymerase can inhibit PCR.

Tables(2.4,2.5 ,2.6) shown common sample types known to contain inhibitors. Other important sources of inhibitors are the material and reagents that come into contact with sample during processing or DNA purification (Wilson, 1997).

Table 2. 4: **Biological Substances and Inhibitors**

Biological Substance	Inhibitor	Comment
Blood	Heme Immunoglobulin G	The amplification of blood samples can be significantly reduced or blocked by natural components of blood, such as heme, and immunoglobulin G. Hemin, a hemoglobin derivative, and its breakdown products, bilirubin and bile salts, are also found to be PCR inhibitors.
Vaginal Samples	Bacteria Microorganisms	Bacteria and microorganisms are commonly found in vaginal, fecal, and buccal samples. Note: these can also be found in other biological samples found at crime scenes.
Buccal Samples		
Fecal Samples		
Hair Tissue	melanin	Melanin, a pigment that affects skin, eye, and hair color, can inhibit PCR.
Bone Teeth	Ca2+	Ca2+ is commonly found in bone and teeth and is known to interfere with Mg2+ concentration, which in turn may affect the activity of Taq polymerase.
Semen	Polyamines	and spermidine (polyamines Spermine originally isolated from semen) are found in ribosomes and living tissues, and can inhibit PCR.
Urine	Urea	Urea, a chemical found in urine, can inhibit PCR.

Table 2. 5: Substrates and Inhibitors

Substrate	Inhibitor	Comment/Example
Textile dyes	Textile dyes	used to color denimIndigo dye
Fabrics	Tannic Acid	Leather
Environmental Samples	compoundsHumic Heavy metals	Soil
Food Constituents	Organic compounds Phenolic compounds Glycogen Fats Ca2+	Many food products, such as milk, contain inhibitors, like Ca2+. However, forensic scientists have had success in developing DNA profiles from saliva left on food or drink containers.

Table 2. 6: Other Inhibitors

Source	Inhibitor	Comment
Extraction Chemicals	Phenolic compounds from the organic extraction,Chelex resin ,Salts,Guanidine,Proteases, Organic solvents,Phosphate buffers Detergents (such as Sodium Dodecyl Sulfate (SDS))	Reagents commonly used in the purification of nucleic acids are inactivators of DNA polymerases. Phenol or Chelex resin left with the extracted DNA can inhibit the PCR process.
Anticoagulants	EDTA and heparin	Known blood reference samples are collected in tubes containing anticoagulants.
Powder	Gloves powder	Many forensics scientists use powdered gloves.
Laboratory plastic ware	PCR tubes	It has been reported that an inhibitory substance can be released from polystyrene or polypropylene upon exposure to ultraviolet light.
Plant and food products	Pollen ,Cellulose ,Plant polysaccharides , Ca2+	Biological material can be deposited on plants and food.

Some tips for avoiding conta mination with PCR reaction in a laboratory setting including

Pre – and post PCR sample processing areas should be separated. Usually a separate room or contaminant cabinet is used for setting up the PCR amplification reactions.

Equipment, such as pipettes and reagent should be separate from other laboratory supply.

Disposable gloves should be worn and changed frequently.

Aerosol, resistant pipette tips should be used and changed on every new sample to prevent cross contamination during liquid transfer.

Reagents should be carefully prepared to avoid presence of any contaminating DNA.

Evidence sample must be analyzed first.

Clean work area and instruments with isopropanol and l or 10 % bleach solution help to ensure the extraneous DNA molecules are destroyed prior to DNA extraction or PCR set up (Kwoka & Higuchi 1989).

2.12.2.7. Advantages of PCR:-

Small amount of DNA may be used .

DNA degraded also amplified.

Large number of copies of specific DNA sequences can be amplified simultaneously with multiplex PCR.

DNA by other than human source such as fungal and bacterial sources will not be amplified, because, human specific primers are used.

Commercial kits are now available for easy PCR reaction setup and amplifications.

2.12 .2. 8. Procedure:-

The PCR usually consists of a series of 20 to 35 repeated temperature changes called cycles; each cycle typically consists of 2-3 discrete temperature steps. Most commonly, PCR is carried out with cycles that have three temperature steps (Figure 8). The cycling is often preceded by a single temperature step (called hold) at a high temperature (>90°C), and followed by one hold at the end for final product extension or brief storage.

Initialization step: This step consists of heating the reaction to a temperature of 94-96°C. It is only required for DNA polymerases that require heat activation by hot-start PCR (Oorschot, 1997).

Denaturation step: Is the first regular cycling event and consists of heating the reaction to 94-98°C. It causes melting of DNA template and primers, yielding single strands of DNA.

Annealing step: The reaction temperature is lowered to 50-65°C allowing annealing of the primers to the single-stranded DNA template. Typically, the annealing temperature is about 3-5 degrees below the Tm of the primers used. (Rychlik, 1990).

Extension/elongation step: The temperature at this step depends on the DNA polymerase used; Taq polymerase has its optimum activity temperature at 75-80°C and commonly a temperature of 72°C is used with this enzyme. The DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand The extension time depends both on the DNA polymerase used and on the length of the DNA fragment to be amplified.

Final elongation: This single step is occasionally performed at a temperature of 60-74°C after the last PCR cycle to ensure that any remaining single-stranded DNA is fully extended.

Final hold: This step at 4-25°C for an indefinite time may be employed for short-term storage of the reaction (Figure 2.8).

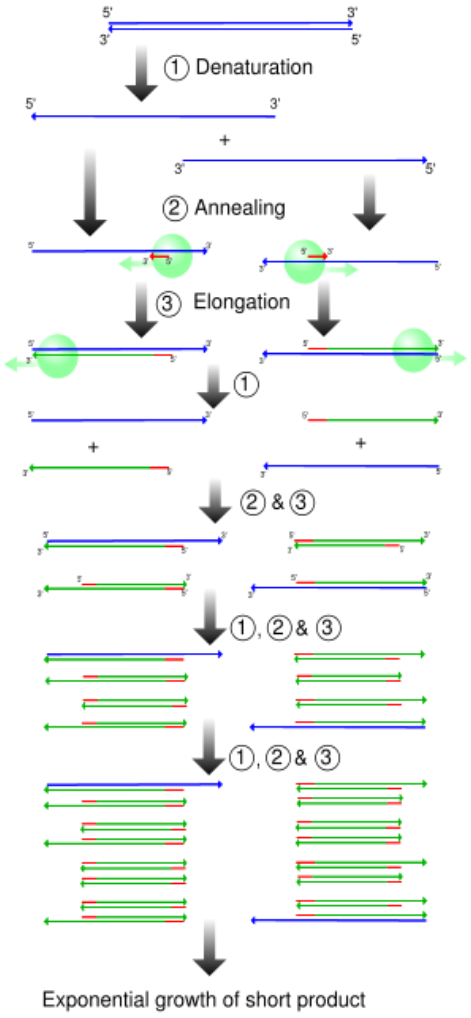


Figure 2.8: Schematic drawing of four PCR cycle. (1) Denaturing at 94-96°C. (2) Annealing at ~65°C (3) Elongation at 72°C.

2.12.3. ABI Prism 310 Genetic Analyzer:-

There are a couple of different methods of separating, detecting, and analyzing a DNA sample, both the underlying principle and the resultant output remain the same. This example will reference an ABI Prism 310 Genetic analyzer module, which uses capillary electrophoresis to separate DNA (Bunch et al., 1997).

The ABI 310 is a single capillary instrument with multiple color fluorescence detection that provides the capability of unattended operation. An operator simply loads a batch of samples in the 'auto sampler' places a capillary and syringe full of polymer solution in the instrument, and start the

'run'. The data and genotype information are serially processed at the rate approximately one sample every 30 minutes of operation. A major advantage of technique for forensic laboratories is that the DNA sample is not fully consumed and may be retested if needed. Once DNA has been extracted, amplified, and then separated according to allele, it must be detected in order to yield useful information to the forensic scientist. Before separation, the mixture of DNA molecules was treated with a mixture of colored, fluorescent dyes. These dyes are specially designed, each to attach to the primers that amplify DNA fragments from particular loci. Each dye is colored differently, and in this way, the DNA fragments from one locus can be distinguished from another locus's DNA fragments, even if the two sets of fragments are otherwise similar, it is not actually the visual color of the dye that is being referred to, but rather the color of light that dye will give off when hit with a lazer of a particular wavelength(Butler, 2015).

2.12.3.1. Components of ABI 310 Analyzer:-

The most commonly used capillary electrophoresis units are manufactured by Applied Biosystems. All Applied Biosystems capillary electrophoresis instruments operate similarly. The two most common models used in forensic science are the ABI 310 and the ABI 3100. This section focuses on the ABI Prism® 310 Genetic Analyzer instrument (Northrop 1993).

- Ions migrate through the capillary during electrophoresis. Positive ions will gather at the cathode and negatively charged ions will gather at the anode. The movement of ions creates an imbalance called buffer depletion. Buffer depletion can impair separation of DNA fragments due to a reduction in current. It is important to replenish or replace the buffer regularly to compensate for buffer depletion.

2.12.3.2. Electrokinetic Injection:-

- This method requires the immersion of the capillary end into the sample. Electrokinetic injection is the only method used in forensic DNA analysis.
- The DNA sample is loaded into the capillary separation matrix by electrokinetic injection. The process consists of the transfer of negatively charged ions via an electromotive force. As current flows from the cathode to the anode, the DNA sample is introduced at the cathode end of the capillary. Because only negatively-charged ions are transferred from the sample in the process, there is no significant loss of sample volume.
- This type of transfer is directly influenced by the ionic strength of the sample (Corstiens, 1996). Ideally, only the DNA present in the sample will contribute to the ionic strength. The presence of competing ions other than DNA rapidly degrades or prevents the injection process.
- When these sample ions enter a region where the polymer solution and buffer are at higher ionic strength, they slow down and stack as a sharp band at the boundary between the sample plug and the electrophoresis buffer (Altira, 2005).

2.12.3.3. Separation:-

- The cathode buffer reservoir, a water reservoir, and a waste vial are located in the autosampler. A short period of electrophoresis injects the sample into the capillary. Next, the autosampler moves the cathode buffer reservoir to the capillary and cathode electrode to continue electrophoresis. The buffer used in the CE system is viscous and adheres to the exterior of the capillary during the buffer fill step. In the subsequent injection step this material could contaminate the sample, change its ionic strength, and reduce the quantity of material

injected. The water reservoir is used to wash the cathode and capillary tip between sample injections.

- The buffer solution moves through the capillary under the influence of an electric field. This phenomenon is termed electroosmotic or electroendosmotic flow. The direction of the electroosmotic flow is toward the positively charged anode, which means that the buffer flows from the source vial, through the capillary, through the detector, to the destination vial. The DNA fragments migrate towards the anode reservoir.
- Performance Optimized Polymers™ dynamically coat the capillary wall to control electroosmotic flow during electrophoresis. The most common polymer used is ABI's Performance Optimized Polymer™-4 (POP-4™).(Lazaruk 1998) This polymer was developed to meet the following specifications:
- detect alleles differing in size by a single base (up to 250 base pairs in length)
- size alleles of the same length with a precision of less than 0.15 nucleotide standard deviation
- require less than 30 minutes analysis time per sample
- provide capillary life of at least 100 injections
- Provide a highly denaturing environment for the DNA samples**.**

2.12.3.4. Fluorescent detection:-

Most CE systems use a form of spectroscopic detection, namely laser-based excitation of the fluorescently labeled DNA fragments. Laser light is directed on to the end of the capillary through the cell window. The laser excites the dyes, so that DNA fragments are illuminated as they pass by this window. The fluorophores primarily used in DNA labeling are dyes that fluoresce in the visible region of the spectrum (approximately 400-600nm) (Butler, 2015).

Each fluorescent dye emits its maximum fluorescence at a different wavelength. The emitted fluorescence is passed through a diffraction grating and is captured by a CCD camera. The grating separates the fluorescence of each dye. The output is sufficiently separated to allow for the analysis of loci which are of similar size but have different fluorescence labels.

- The precise spectral overlap between the dyes is measured by running DNA fragments labeled with each of the dyes in separate CE injections, producing a matrix file. The matrix file allows for multi-component analysis.
- The response from the detector is measured in relative fluorescent units (RFU). The output from the instrument is represented as peaks in an electropherogram with an x and y axis.
- The x-axis is a measure of time plotted as scan data points and the y-axis is plotted as RFUs.

2.12.3.5. Data analysis:-

The steps for assigning an allele call to each peak are, data collection, peak recognition, color separation, peak sizing allelic ladder comparison and allele assignment (i.e., genotype), DNA fragments, represented as peaks on an electropherogram, can be sized relative to the internal size standard (ISS), which is mixed in with the amplified product.

For forensic short tandem repeat (STR) testing, allele sizes must have a sizing precision of a single base. Variables such as temperature, sample and buffer ionic strengths, osmotic flow, and electric field can all influence DNA mobility. The DNA fragments are sized by comparing their migration time with that of flanking internal standard peaks (Figure 2.9). The size is then calculated by interpolation, assuming a linear relationship between peaks (Butler, 2015).

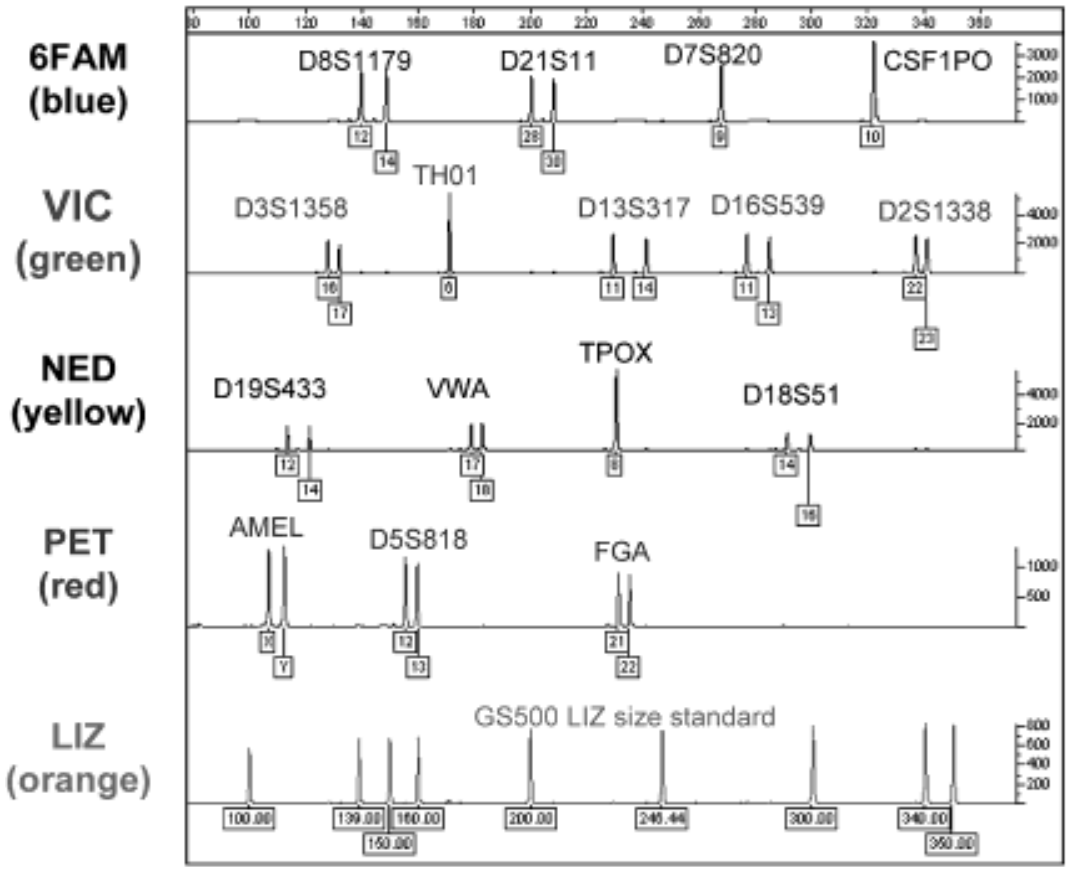


Figure 2. 9: Good resolution due to AmpFlSTR Identifier STR kit (5-dyes).

Sequence variation and attachment of dyes affect the size of the fragments. The size of an individual fragment is determined by comparison with the internal size standard. The allelic designation is determined by comparing its assigned size with the allelic ladder. This ISS is labeled with a different colored dye so that it can be distinguished from the DNA fragments in the sample. The ISS generally contains DNA fragments spanning the size range.

ABI uses three software programs for the genotyping process:-

- GeneScan®
- Genotyper®
- GeneMapper® ID

GeneScan® software spectrally resolves the dye colors for each peak and is used to size the DNA fragments in each sample. This data is then imported in the Genotyper® program, which compares the sizes of alleles in the allelic ladder to those obtained for each sample. GeneMapper® ID is a program that was released by Applied Biosystems (AB) in 2012, and which combines the functions of GeneScan® and Genotyper®.

2.12.3.6. Sample preparation:-

The samples are mixed with formamide and an internal size standard. Formamide can be purchased from multiple vendors and varies in quality. High quality formamide is important for successful capillary electrophoresis.

Formamide: to effectively separate the amplicons. Double-stranded DNA can be modified to single-stranded DNA using formamide.

2.13. STR kits:-

A number of kits are available for multiplex PCR amplification of STR Marker used in DNA Typing. Two primary vendors for STR Kits used by the forensic DNA community exist, the Promega Corporation and applied Bio system. Recently Companies have begun offering commercial STR Kits Appendix (table 2).

3. Material and Methods

From about (35) different cases, samples were collected from different sources. Samples were treated and tested inside and outside Sudan by different means depending on its type and purpose as followed.

3.1. Materials

Fifteen paternity cases, peripheral blood (1ml in EDTA tube) were taken from ten paternity cases, which consist of 32 samples collected from mother, one child and one or more alleged father(s) .Whereas, 5 cases consist of 16 blood sample collected in FTA card Table (3.1).

Ten sexual assault cases were analyzed from different evidences (suspect‹s clothes, blood taken from suspect (represent reference sample), sheet, swab from victim‹s blood, victim‹s clothes, and blood taken from victim Table (3.2) .

Ten murder cases were also analyzed from different evidences (suspect‹s clothes, blood taken from suspect, suspect‹s car, suspect‹s knife) (represent reference sample), sheet, swab from victim‹s blood , victim‹s clothes, and blood taken from victim, Table (3.3).

Table (3.1) Paternity cases & evidences

Case NO	Type	Evidences (number & Type)				
Case 1	paternity	1A: FTA card	1B: FTA card	1C: FTA card		
Case 2	Paternity	2 A: FTA card	2B: FTA card	2 C: FTA card		
Case 3	Paternity	3 A: FTA card	3B: FTA card	3 C: FTA card		
Case 4	Paternity	4 A: FTA card	4B: FTA card	4 C: FTA card		
Case 5	Paternity	5 A: FTA card	5B: FTA card	5 C: FTA card		
Case 6	Paternity	6 A: liquid blood	6B: FTA card	6C: liquid blood		
Case 7	Paternity	7A :liquid blood	7B: liquid blood	7C: liquid blood		
Case 8	Paternity	8A: liquid blood	8B: liquid blood	8C: liquid blood		
Case 9	Paternity	9A: liquid blood	9B: liquid blood	9C: liquid blood		
Case 10	Paternity	10A: liquid blood	10B: liquid blood	10 C: liquid blood		
Case 11	Paternity	11A: liquid blood	11B: liquid blood	11C :liquid blood		
Case 12	Paternity	12A: liquid blood	12B: liquid blood	12C: liquid blood		
Case 13	Paternity	13A: liquid blood	13B: liquid blood	13C :liquid blood		
Case 14	Paternity	14A: liquid blood	14B: liquid blood	14C:liquid blood	14D: liquid blood	
Case 15	Paternity	15A: liquid blood	15B:liquid blood	15C: liquid blood	15D:liquid blood	15E: liquid blood

Table (3.2) Rape cases & evidences

Case NO	Type	Evidences (number & Type)			
Case 16	Rape	16A:cloth(sperm)	16B: FTA card	16C: Sheet	
Case 17	Rape	17A : (child clothes)	17B: Suspect Blood FTA card		
Case 18	Rape	18A: victim cloth	18B: Suspect Blood FTA card		
Case 19	Rape	19A:swab	19B:liquid blood	19C: cloth	
Case 20	Rape	20A:swab	20B:liquid blood	20C : cloth	
Case 21	Rape	21A: cloth	21B:liquid blood		
Case 22	Rape	22A liquid blood	22B liquid blood	22C liquid blood	22D:liquid sample
Case 23	Rape	23A:liquid blood	23B:liquid blood	23C:swab	23D:cloth
Case 24	Rape	24A:liquid blood	24B:liquid blood	24C:swab	
Case 25	Rape	25A:liquid blood	25B:liquid blood	25C:Liquid sample	

Table (3.3) Crime Scene cases & evidences

Case NO	Type	Evidences (number & Type)					
Case 26	Crime Scene	26A: blood stain (swab)			26B: blood stain (cloth)		
Case 27	Crime Scene	27A: blood stain (cloth)			27B: blood stain (cloth)		
Case 28	Crime Scene	28A: blood stain (cloth)			28B: blood stain		
Case 29	Crime Scene	29A: blood stain(cloth)			29B: blood stain (cloth)		
Case 30	Crime Scene	30A: blood stain (cloth)			30B: blood stain (cloth)		
Case 31	Crime Scene	31A:Blood Stain	31B:clothes		31C:Liquid blood .vi	31D: Liquid blood. suspect	
Case 32	Crime Scene	32A: blood stain.car (Cotton)			32B: blood stain. victim (Cotton)		
Case 33	Crime Scene	33A:liquid blood	33B:Knife (Stain)		33C:Cloth(suspect)		
Case 34	Crime Scene	34A: blood Stain. paper	34B: Shoes	34C: clothes	34D: Clothe	34E: liquid blood	34F:Soil sample
Case 35	Crime Scene	35 A: blood stain (swab)			35B: blood stain (Knife)		

3.2. Identifications of samples:-

3.2.1.Classical test:-

3.2.1.1. Detection of liquid blood and blood stain:-

Many ways used to detect human blood and blood stain

3.2.1.1.1. Benzidine test (presumptive test) :-

was done which includes the samples below: (26A, 26B, 27A, 27B, 28A, 28B, 29A, 29B, 30A, 30B, 31A, 31B, 32A, 32B, 33B, 33C, 34A, 34B, 34C, 34D, 34F,35A,and 35B) as mention in Table (3.3).

Procedure:-

Small piece from the portion suspected stain with blood "or one drop of liquid sample" was placed on filter paper (What man cellulose filter paper). A drop of benzidine solution was put over it, followed by drop of conc H_2O_2. Blue color was observed which indicated the presence of hemoglobin.

3.2.1.1.2. Anti human test kits (confirmatory test):-

was done which includes the samples below:

(26A, 26B, 27A,27B, 28A, 28B, 29A, 29B, 30A, 30B, 31A, 31B, 32A,32B, 33B, 33C, 34A, 34B, 34C, 34D,34F, 35A,and 35B) as mention in table 13 .

Procedure:-

Small piece of the suspected blood stain (or one drop of liquid sample) was placed on 1.5 ml tube. The buffer solution was added to the sample and shacked. One drop of solution was put inside the small well on the test kit. Wait for the buffer solution to diffuse across the kit. Two lines were observed which indicate the presence of human blood .

3.2.1.2. Detection of semen or seminal fluid:-

3.2.1.2.1: Physical Examination:-

Seminal stain are starchy, yellowish white, thick, glary, opalescent have specific odor, under UV light the florescence of seminal stain is a bluish white color.

3.2.1.2.2. Prostate specific antigen test (PSA Kit):-

Small piece of sample stain (or one drop of liquid sample) was placed on 1.5 ml tube. The buffer solution was added to the sample and then shaken. One drop of solution was put inside the small well on the test kit .Waited for1 minute, the buffer solution diffuse across the kit. Two lines were observed which indicate the presence of human seminal fluid .

3.2.1.2.3: Acid phosphate test:-

Was done for the samples below:
(16A, 16C, 17A, 18A, 19A, 19C, 20A, 20 C, 21A, 22D, 23C, 23D, 24C, and 25 C) as mention in Table (3.2).

A small piece of suspected seminal stain material (or one drop of liquid sample) was placed on 1.5 ml tube. A few drops of distilled water were added to cover the sample and the tube was Stoppard, then vortexed for 10 seconds. One drop was pipetted and placed on the whatman filter paper or other suitable test paper . 1-2 drops of reagent A were added for 30 seconds (no colors appear). followed by 1-2 drops of reagent B, violet color was observed which indicated the presence of seminal fluid.

3.2.1.2.4. Microscopic examination (confirmatory test):-

Was done for the samples below:-
(16A, 16C, 17A, 18A, 19A, 19C, 20A, 20C, 21A, 22D, 23D, 23C , 24C, and 25C) as mention in Table (3.2) .

The solution prepared above "containing the sample" were transferred to another tube, and centrifuged for 30 seconds to collect the sperms in the button solution (if present). The button solution was pipetted and placed on

a clean-labeled microscope slide, and then fixed in 56° C oven for 30 minutes. The slide was stained with a few drops of Eosin dye for 10 minutes, and then washed by water. The slide was stained with a few drops of Malackite green dye for 10 minutes and washed by water and allowed to dry. Examined under microscope to observe sperms, and continued to extract the DNA from the sample.

3.2.2. Molecular test:-

3. 2.2.1. Extraction of DNA from samples:-

3. 2.2.1.1.Organic method: -

3. 2.2.1.1.1. Phenol-chloroform isoamyl alcohol (PCI) method:

(For blood, blood stain or semen stain) was done for the samples below: (16B, 17B, 18B, 19B, 20B, 24C1, 26A, 26B, 27A, 27B, 28A, 28B, 29A, 29B, 30A, and 30B) as mention in Table (3.2)& Table (3.3).

200 µl of blood sample were placed in a 1.5 ml tube "small piece of sample stain". 500 µl of stain extraction buffer (lysis buffer for liquid blood), and 15 µl of protenase K were added (in blood stain and seminal stain samples , 20 µl of DTT were added) followed by vortex . The samples were incubated at 56°C for one hour (seminal stain incubated over night).

The sample was transferred to another tube (the fabric material was removed from the sample). 500 µl of phenol-chloroform isoamylalcohol (PCI) were added and vortexed for 5 seconds, and centrifuged at 14,000 rpm for 5 minutes. The supernatant was taken carefully and an equal volume of absolute alcohol was added (chilled alcohol at - 20° C) , and kept at -20°C for 30 minutes, then centrifuged for 15 minutes at 14,000 rpm. The supernatant was decanted and IM 70% ethanol was added. The tube was inverted several times until the white threat of DNA is appeared, and then centrifuged to precipitate DNA . The supernatant was decanted. The tube was inverted in filter paper, and

dissolved in 50 µl TE buffer. The samples were kept in refrigerator or directory to PCR (Lori s et al.,2005).

3.2.2. 1.1. 2. Differential extraction of DNA from seminal stain: - was done for samples : (16A ,16C,17A ,18A,19C,20C, 21A, and 24C2) as mention in Table (3.2).

3mmX3mm of the seminal stain that had been checked before for the presence of sperms has been cut, and Put in an eppendorf tube (1.5 ml), 500 µl digest buffer was added followed by 15 µl proteinase K and vortexed. Samples were put in 56°c in water bath for 1-2 hours. Samples were centrifuged for 3 minutes at 14,000 rpm to collect the sperms in the button solution. The supernatant was taken out and digest buffer was added to the pellet and centrifuged (for washing), then the supernatant was taken out. The pellet was washed with dH_2O to get rid of the supernatant and then 500 µl of stain extraction buffer and 15 µl proteinase K and 5 µl 1M DTT were added, and followed by vortex. The sample was incubated at 56°C in water bath for overnight. The tube was taken and 500 µl (PCI) was added to it, vortexed, and then centrifuged for 5 minutes at (14,000 rpm). The supernatant was taken carefully and an equal volume of absolute alcohol was added, and kept at -20°C for 30 minutes, then centrifuged for 15 minutes at 14000 rpm. The supernatant were decanted and IM 70% ethanol was added. The tube was inverted several times until the white threat of DNA appeared, and then centrifuged to precipitate DNA. The supernatant was decanted. The tube was inverted in filter paper, and dissolved in 50 µl TE buffer .The samples were kept in refrigerator or directory to PCR(K Yoshida, et al.,1995).

3.2.2.1. 2.Chelex extraction from whole blood, bloodstain: (inorganic method):-

The above method was done which includes the samples: (1A, 1B, 1C, 2A, 2B, 2C, 3A, 3B, 3C, 4A, 4B, 4C, 5A, 5B, 5C) as mention in Table (3.1).

200 µl whole blood or blood stain approximately 3x3 mm was placed in 1.5 ml microtube.1ml sterile water was pipetted into the tube and vortexed for 5 seconds. Sample was Incubated at room temperature for 30 minutes, and mixed occasionally by inversion or generate vortexing. Sample was centrifuged at 14,000 rpm for 5 minutes. The supernatant was removed and discarded, leaving enough to cover the pellet without disturbing it (if the sample is a blood stain, the fabric substrate left in the tube with the pellet). 200 µl of 5% chelex, was added and vortexed. The sample was incubated at 56°C for 30 minutes. The fabric material was removed from the solution (in blood stain). The solution was vortexed for 10 seconds and incubated at 100°C for 8 seconds, vortexed for 10 seconds .The sample was centrifuged at 14,000 rpm for 2 minutes. The sample then was ready for PCR amplification process (JM. Polski et al., 1998).

3.2.2.1.3. Extraction by Maxwell™ 16 automated DNA purification instrument:-

3.2.2.1.3.1. Extraction of whole blood:-

This was done to the samples below:

(6 A, 6C, 7A, 7B, 7C, 8A, 8B, 8C, 9A, 9B, 9C, 10A, 10B, 10 C, 11A, 11B,11C,12A,12B,12C,13A, 13B, 13C, 14A, 14B, 14C, 14D, 15A, 15B, 15C, 15D,15E,21B,22A,22B,22C,23A,23B,24A,24B,25A,25B,31C,31D, 33A, and 34E) as mention in Table(3.1) ,Table(3.2) & Table(3.3): axwell purification kits (cat #AS1200) was used (SEV system).

200 µl of liquid blood sample was Pipetted and transferred into well #I. (Well #I is the well closest to the cartridge label and furthest from the user). One plunger was placed into well #7 of each cartridge (well #7 is the well closest to the ridged side of cartridge).

The cartridge was transferred from cartridge preparation rack to the Maxwell™ 16 platform. Blue Elution tube was placed into the elution tube slots at the front of the plate form. 300 µl of elution buffer was added to each elution tube. The DNA extracted was transferred from elution tube to 1.5 ml tube. The sample was diluted using TE buffer (1:80), and kept in refrigerator or directory to PCR (Applied Biosystems 2012).

. 3.2.2.1.3.2. Extraction of DNA from other liquid sample:-

This was done to samples 22D & 25C as mention in Table (3.2).

250 µl of liquid sample were pipetted in a tube. 500 µl lysis buffer containing DTT (100:1) were added and in trace liquid sample 15 µl protenase K was added. After vortex sample was incubated at 56°C for one hour, and vortexed briefly. The sample was transferred into well #I

3.2.2.1.3.3. Extraction of DNA from blood stain:-

The above technique was done to the samples below :

(6B, 31A, 31B,32B,33B,33C, 34A, 34C, 34D, and 34F , 35A, as mention in Table (3.1), Table (3.2) and Table (3.3).

(DNA IQ™ Reference sample kit (cat #AS1200) was used "SEV system") Small piece of the sample has been cut and put in a labeled 1.5 ml microtube. 500 µl of lysis buffer containing DTT (500:5), and 15µl protenase K were added, and vortexed briefly. The sample was incubated at 56°C for 30 minutes. The tube was briefly vortexed to recover any evaporated liquid on the sides of the tube. The sample was transferred to a labeled fresh 1.5 ml tube (the fabric material was removed from the solution), and Centrifuged at 14,000 rpm at room temperature for 2 minutes.The supernatant was taken and transferred to well #I. Diluted DNA extracted by adding TE buffer (1:4)

3.2.2.1.3.4. Extraction of DNA from semen stain:-

The above was done to samples 19A, 20A, 23C, and 23D, as mention in Table (3.2), **(**DNA IQ™ Reference sample kit(cat #AS1200),was used

"SEV system") Each semen stain was placed into labeled 1.5 ml microtube. 160 µl lysis buffer, 20 µl of protenase K solution and 20 µl of IMDTT were added. Each tube was vortexed for 10 seconds, incubated overnight at 56°C, and then vortexed for 10 seconds. The sample was transferred to another tube. 400 µl (two volumes) of lysis buffer was added. Each sample was vortexed, and centrifuged at 25°C for 2 minutes. The supernatant was transferred to well #I. 20-30 µl of elution buffer were added to each elution tube. The DNA extracted was diluted by adding TE buffer (1:4)

3.2.2.1.3.5. Extraction of DNA from traces samples:-

This was done to 32A, 34B, 35B, sample.

(The DNA IQ™ case work sample kit (cat #AS1250) with the Maxwell 16 LEV instrument was used) Each sample was placed in labeled 1.5 microtube. 500 µl of lysis buffer, 5 µl of DTT and 15 µl protenase K (in liquid sample 200 µl of lysis containing DTT(Dithiothreitol) to 100 µl of sample) were added, and vortexed . The sample was incubated at 56°C for 30 minutes, and then vortexed. The sample was transferred into another fresh tube. The fabric material was removed from the solution, and Centrifuged at room temperature for 2 minutes . The supernatant was transferred to well #I. 20 µl of elution buffer was added to each elution tube.

3.2.2.2. Amplification of DNA: PCR techniques:-

The DNA was amplified by the thermal cycling conditions (Table 3.4).
Table 3.4: Thermo cycler program

Initial incubation step	Cycle (30 cycles)			Final extension	Final step
	Denature	Anneal	Extend		
95°C	95°C	59°C	72°C	60°C	4 - 25°C
11 min	1 min	1 min	1 min	60 min	Forever

3.2.2.2.1. PCR techniques:-

Labeled tube was prepared for each sample. The master mix was vortexed for 5 seconds. 15 µl of master mix was placed to each labeled PCR tube. 10 µl of sample was Added to the PCR tube (each sample in specific tube). 10 µl of positive control was added to the positive control tube. 10 µl of TE buffer "negative control" was added to the labeled negative control tube. The tubes were centrifuged to remove any liquids from the caps . All tubes were transferred to the PCR machine (Gene Amp-9700 thermal cycler) and begin to run (Applied Biosystems 2012).

3.2.2.3. Identification of DNA: ABI 310Genetic Analyzer

Labeled tube for each sample was prepared. 25µL of mixture (HIDI formamide and Gene Lize 500) solution was aliquoted to the tube. 1.5µL of PCR product or Allelic ladder was added and mixed by pippeting up and down. Each sample was vortexed and spined. Each sample denatured for 3 minutes at 95°C. Each tube was chilled at 3 minutes on ice. The samples were put in the sample tray, then in the autosampler and began to run (Applied Biosystems 2012).

3.2.2.4. Statistical Analysis:

Descriptive statistics, Chi-square test and chart was done by using spss (statistical package for social sciences),version 11.5, to assess the DNA-based evidence as discrimination for criminal justice .

4.1. Classical techniques:

4.1.1. Benzidine Test:

When Benzidine was added, appearance of green bluish colored indicated of appearance of blood ,should appear by (Figure 4.1).

4.1.2. Antihuman Test Kits:

Positive result was shown by two pink lines. Negative result was shown by only one pink band, (Figure 4.2).

4.1.3. Acid Phosphate (AP) Semen Detection Test:

A ppearance of violet color indicated to the presence of spermatozoa. (Table 4.2).

3.1.4. PSA Quick Test Kits:

This result gave a positive result indicated by presence of two lines. (Figure 4.4).

Table (4.1): Shows Identification of Human Blood Using Benzidine Test & Antihuman Test Kits

(Dry Blood)

Case .No	Evidence .No	Benzidine Test	Antihuman Test Kits
Case26	26 A	+	+
	26 B	+	+
Case 27	27 A	+	+
	27 B	+	+
Case 28	28 A	+	+
	28 B	+	+
Case 29	29A	+	+
	29 B	+	+
Case 30	30 A	+	+
	30 B	+	+
Case 31	31 A	+	+
	31B	+	+
Case 32	32 A	+	+
	32 B	+	+
Case 33	33B	+	+
	33C	+	+
Case 34	34A	+	+
	34B	+	+
	34C	+	+
	34D	+	+
	34F	+	+
Case 35	35A	+	+
	35B	+	+

Figure 4.1(Benzidine test)

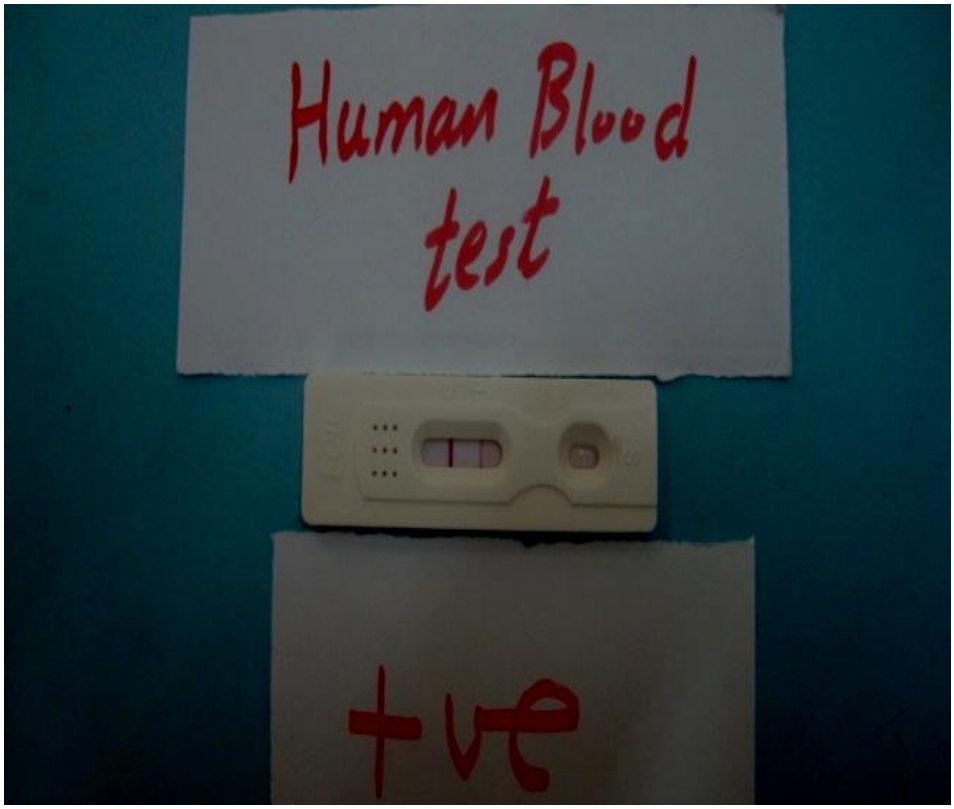


Figure 4.2 (Positive Human Blood test)

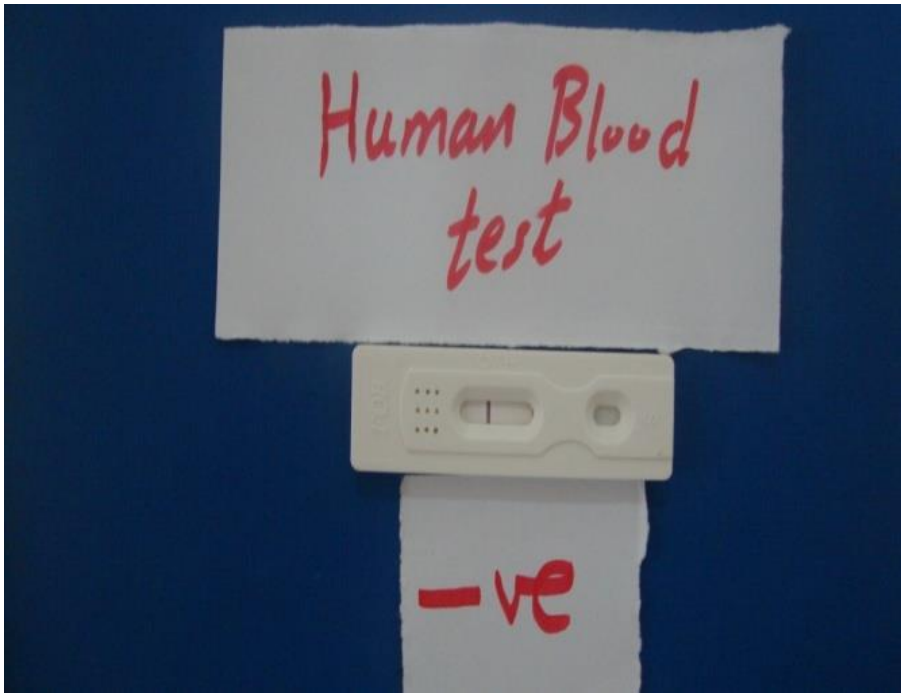


Figure 4.3 (Negative Human Blood test)

Table (4.2): Shows Identification of Seminal fluid.

(Classical tests demonstrates)

Case .No	**Evidence .No**	Acid Phosphotase Test	PSA Quick Test Kits	Microscopic Examination
Case 16	16A	+	+	+
	16C	+	+	+
Case 17	**17A**	+	+	+
Case 18	**18A**	+	+	-
Case 19	**19A**	+	+	-
	19C	+	+	+
Case 20	**20A**	+	+	-
	20 C	+	+	+
Case 21	**21A**	+	+	-
Case 22	**22D**	+	+	+
Case 23	**23C**	+	+	-
Case 23	**23D**	+	+	+
Case 24	**24C**	+	+	+
Case 25	**25 C**	+	+	+

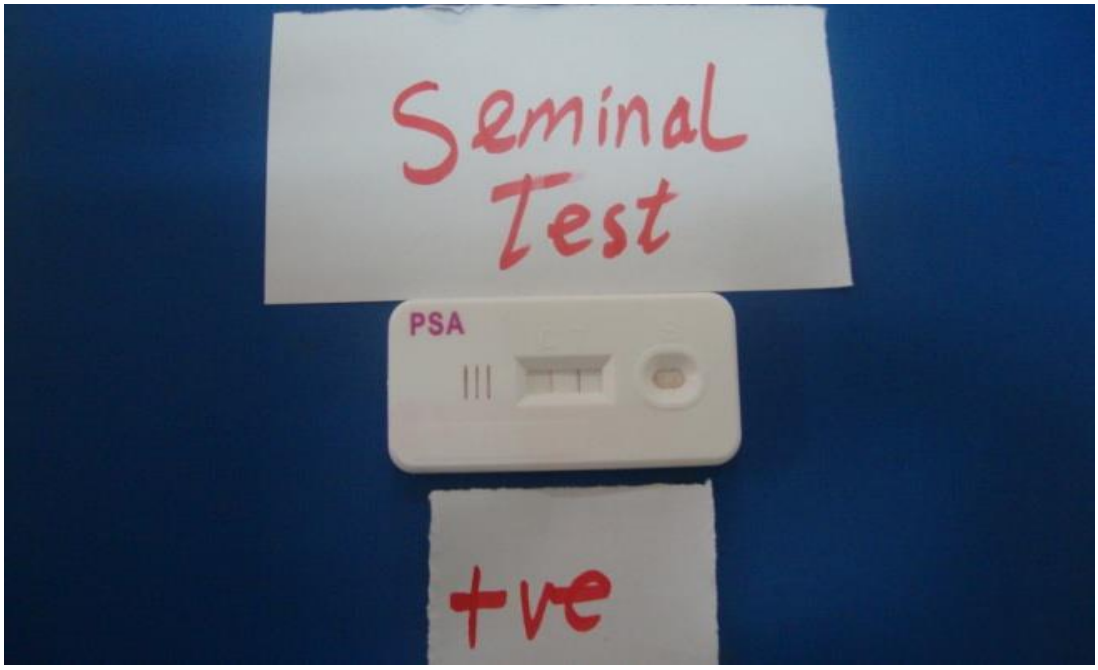


Figure 4.4 (Positive Seminal test)

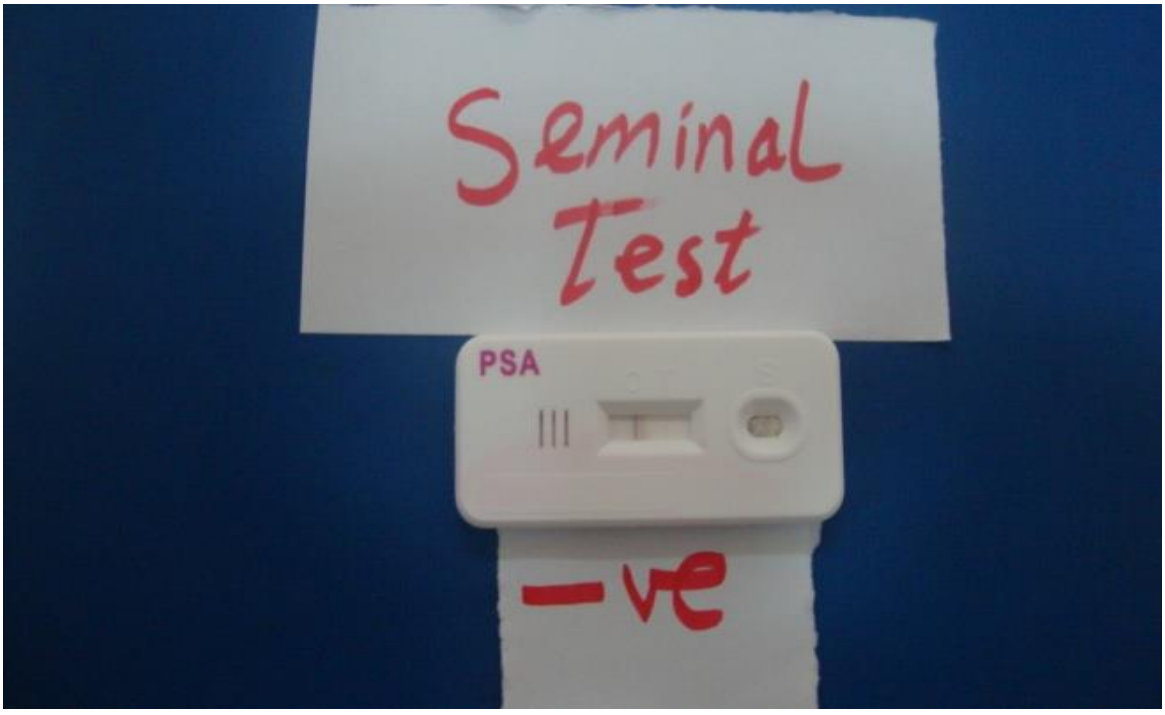


Figure 4.5 (Negative Seminal test)

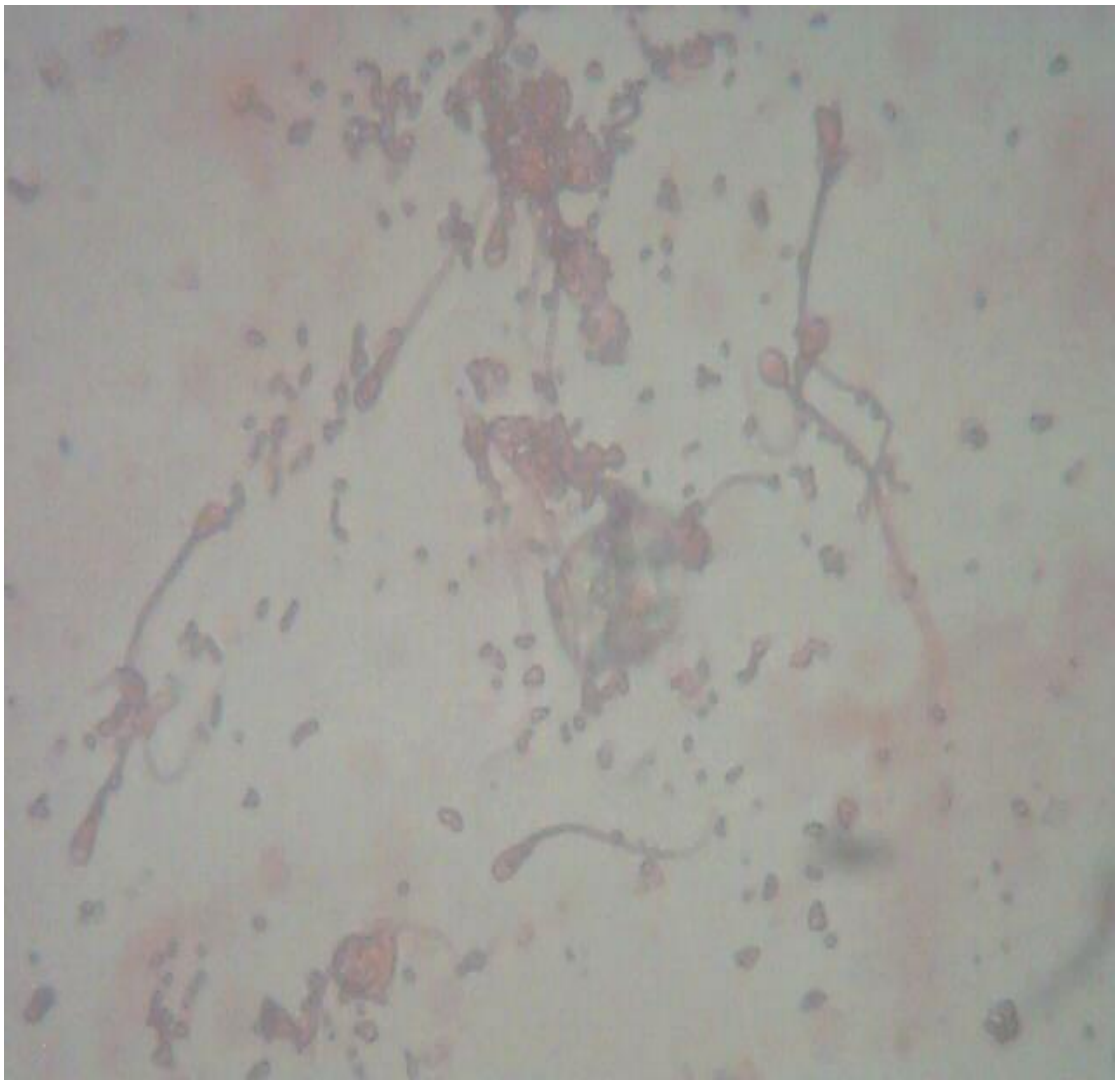
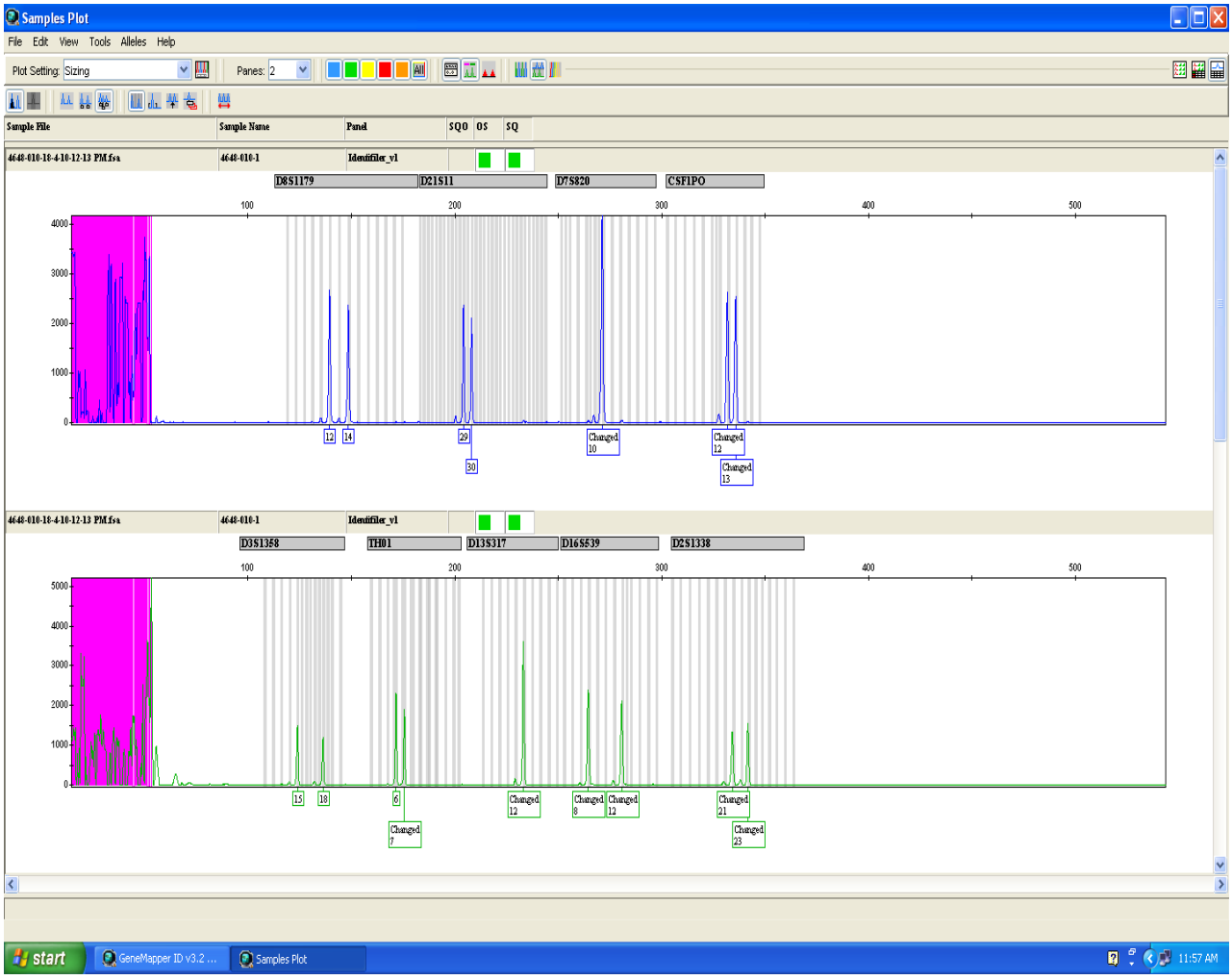


Figure 4.6 (Microscopic Test)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File Edit View Tools Alleles Help
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D8S1179
D21S11
D7S820
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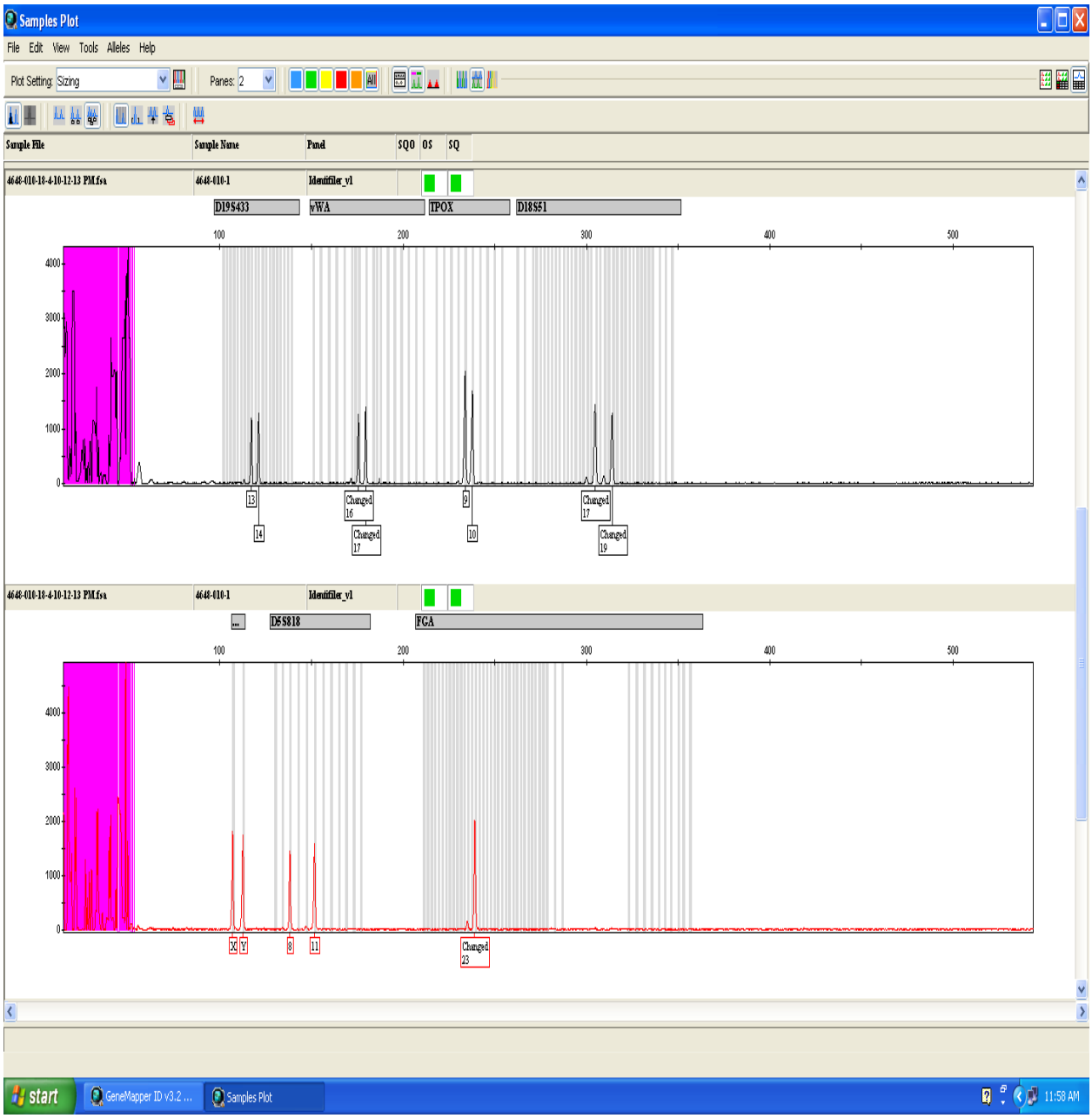


Figure 4.7: Shows the Victim's DNA from Victim's clothes Case no (21A)

4.2. Molecular techniques

4.2.1. Paternity test:

(15) Paternity cases, contained (17) samples had been taken from alleged father are performed where the identity of the father of a child is in dispute. These cases involve the mother, the child and one or more alleged father. By comparing half of genotype with the mother and half with alleged father, and comparison, found that (11) cases confirmed by their father to the child (inclusion), and (4) cases denied their father (exclusion)Tables (4.3 to 4.17) .

4.2.2. Rape test:

(10) Rape cases were compared to suspect's DNA on victim, victim's body or clothes, (5) cases were matched (inclusion), (4) cases were not matched (exclusion) and (1) was contaminated Tables (4.18 to 4.27) .

4.2.3. Murder test:

Murder cases, the suspect's DNA profile were compared to some evidences collected from the crime scene, which led to identify the criminal, and link between victim's DNA on object in suspect's possession Tables (4.28 to 4.37) .

Table (4.40) shows the Author's DNA profiling tested in Sudan, and table (4.41) shows the Author's DNA profiling tested in Jordan.

Table 4.3:Shows The 16 Core set of STR Loci Case No.1 (Paternity)

NO	Genetic loci	1A: Alleged Father		1B: Mother		1C : child	
		Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
1	**D8S1179**	12	16	12	14	12	14
2	**D21S11**	30	31.2	28	30	28	30
3	**D7S820**	8	10	8	11	8	10
4	**CSF1PO**	10	12	11	12	11	12
5	**D3S1358**	16	16	15	17	16	17
6	**TH01**	7	8	7	7	7	7
7	**D13S317**	12	13	11	12	11	12
8	**D16S539**	13	13	9	9	9	13
9	**Penta E**	7	13	8	14	8	13
10	**Penta D**	5	11	8	10	10	11
11	**VWA**	16	19	17	18	16	17
12	**TPOX**	6	10	9	11	10	11
13	**D18S51**	15	18	16	19	15	16
14	**D5S818**	12	12	11	11	11	12
15	**FGA**	19	19	19	19	19	19
16	**AMELOG.**	X	Y	X	X	X	X

Table 4.4 :Shows The 16 Core set of STR Loci Case No.2 (Paternity)

NO	Genetic loci	2A: Alleged Father		2B: Mother		2C : child	
		Allele 1	**Allele 2**	**Allele 1**	**Allele 2**	**Allele 1**	**Allele 2**
1	**D8S1179**	13	14	14	15	13	15
2	**D21S11**	29	35	29	29	29	35
3	**D7S820**	8	9	8	9	8	8
4	**CSF1PO**	10	12	11	12	10	12
5	**D3S1358**	15	16	15	17	16	17
6	**TH01**	6	9	7	7	7	9
7	**D13S317**	12	12	12	13	12	13
8	**D16S539**	8	13	10	12	10	13
9	**Penta E**	13	18	12	15	12	18
10	**Penta D**	6	11	11	12	6	11
11	**VWA**	15	17	14	15	14	15
12	**TPOX**	8	11	9	9	9	11
13	**D18S51**	14	17	17	19	14	17
14	**D5S818**	13	13	8	12	12	13
15	**FGA**	23	27	22	28	27	28
16	**AMELOG.**	X	Y	X	X	X	Y

Table 4.5:Shows The 16 Core Set of STR Loci Case No.3 (Paternity)

NO	Genetic loci	3A: Alleged Father		3B: Mother		3C : child	
		Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
1	**D8S1179**	12	14	14	14	14	14
2	**D21S11**	30	32.2	28	30	28	30
3	**D7S820**	10	10	10	13	10	13
4	**CSF1PO**	11	13	10	11	11	13
5	**D3S1358**	16	16	16	17	16	17
6	**TH01**	6	9	7	7	6	7
7	**D13S317**	8	12	12	13	12	13
8	**D16S539**	10	11	11	12	11	11
9	**Penta E**	16	18	8	13	8	16
10	**Penta D**	2.2	14	9	16	2.2	9
11	**VWA**	17	17	14	17	14	17
12	**TPOX**	8	8	9	12	8	9
13	**D18S51**	15	19	15	18	15	19
14	**D5S818**	8	13	8	11	8	8
15	**FGA**	22	25	23	25	22	23
16	**AMELOG.**	X	Y	X	X	X	Y

Table 4.6 :Shows The 16 Core Set of STR Loci Case No.4 (Paternity)

NO	Genetic loci	4A: Alleged Father		4B: Mother		4C : child	
		Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
1	**D8S1179**	14	14	13	13	13	14
2	**D21S11**	31	33.2	28	29	28	31
3	**D7S820**	8	10	10	10	8	10
4	**CSF1PO**	11	13	7	10	10	13
5	**D3S1358**	16	17	15	17	17	17
6	**TH01**	7	7	7	8	7	8
7	**D13S317**	8	13	11	12	11	13
8	**D16S539**	9	12	9	12	9	12
9	**PENTA E**	8	16	8	14	8	16
10	**PENTA D**	2.2	11	2.2	7	2.2	11
11	**VWA**	15	18	15	16	15	16
12	**TPOX**	8	9	8	11	9	11
13	**D18S51**	18	18	18	19	18	19
14	**D5S818**	8	12	11	12	8	12
15	**FGA**	22	24	21	22	22	24
16	**AMELOG.**	**X**	**Y**	**X**	**X**	**X**	**X**

Table 4.7:Shows The 16 Core Set of STR Loci Case NO.5 (Paternity)

NO	Genetic loci	5A: Alleged Father		5B: Mother		5C : child	
		Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
1	**D8S1179**	13	15	11	14	11	17*
2	**D21S11**	30	34	29	32.2	29	30
3	**D7S820**	8	12	8	8	8	10*
4	**CSF1PO**	11	12	12	12	11	12
5	**D3S1358**	16	18	16	17	17	17*
6	**TH01**	7	7	7	7	6*	7
7	**D13S317**	11	13	8	11	11	13
8	**D16S539**	11	11	11	12	9*	11
9	**D2S1338**	7	21	15	17	15	18
10	**D19S433**	2.2	12	8	11	11*	11
11	**VWA**	14	17	15	16	16	17
12	**TPOX**	8	11	8	9	9	10*
13	**D18S51**	16	17	12	14	14	16
14	**D5S818**	12	12	11	13	12	13
15	**FGA**	20	23	20	28	20	25*
16	**AMELOG.**	X	Y	X	X	X	X

*refer to non matching allele

Table 4.8:Shows The 16 Core Set of STR Loci Case No.6 (Paternity)

NO	Genetic loci	6A: Alleged Father		6B: : Mother		6C : child	
		Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
1	D8S1179	13	13	12	14	12	13
2	D21S11	29	29	29	33.2	29	29
3	D7S820	10	10	9	10	9	10
4	CSF1PO	12	12	10	12	10	12
5	D3S1358	16	18	15	17	17	18
6	TH01	6	7	7	7	7	7
7	D13S317	10	12	12	12	12	12
8	D16S539	9	9	8	13	9	13
9	PENTA E	20	22	22	22	22	22
10	PENTA D	14	15.2	13	14	14	14
11	VWA	14	15	16	18	15	18
12	TPOX	8	9	8	10	8	10
13	D18S51	15	16	17	19	16	17
14	D5S818	12	12	11	13	11	12
15	FGA	22	23	24	28	23	28
16	AMELOG.	X	Y	X	X	X	Y

Table 4.9:Shows The 16 Core Set of STR Loci Case No.7 (Paternity)

NO	Genetic loci	7A: alleged father		7B: Mother		7C : child	
		Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
1	**D8S1179**	11	15	14	15	12*	15
2	**D21S11**	29	36	29	30	30*	30
3	**D7S820**	8	10	8	10	8	10
4	**CSF1PO**	11	12	12	13	10*	12
5	**D3S1358**	16	17	16	17	17	17
6	**TH01**	7	7	7	10	6*	7
7	**D13S317**	12	12	12	12	12	12
8	**D16S539**	11	11	11	11	11	11
9	**D2S1338**	19	20	16	20	16*	16
10	**D19S433**	12	14	12	13	12	14
11	**VWA**	16	17	15	17	15	16
12	**TPOX**	9	9	8	9	8	8
13	**D18S51**	12	22	15	22	15	19*
14	**D5S818**	11	13	10	11	7*	10
15	**FGA**	22	28	22	24	23*	24
16	**AMELOG.**	X	Y	X	X	X	X

*refer to non matching allele

Table 4.10:Shows The 16 Core Set of STR Loci Case No.8 (Paternity)

No	Genetic Loci	8A:Alleged Father		8B : Mother		8C : Child	
		Allele1	Allele2	Allele1	Allele2	Allele1	Allele2
1	**D8S1179**	12	13	12	15	12	13
2	**D21S11**	29	30	29	33.2	29	29
3	**D7S820**	10	10	8	9	8	10
4	**CSF1PO**	10	12	9	12	10	12
5	**D3S1358**	17	17	15	17	17	17
6	**TH01**	7	9	6	8	6	9
7	**D13S317**	12	12	13	13	12	13
8	**D16S539**	11	12	10	13	12	13
9	**D2S1338**	20	21	19	19	19	21
10	**D19S433**	13	16.2	11.2	14	11.2	16.2
11	**VWA**	14	16	15	19	15	16
12	**TPOX**	9	11	8	11	11	11
13	**D18S51**	16	17	15	17	17	17
14	**D5S818**	11	12	12	12	12	12
15	**FGA**	21	23	25	30	23	25
16	**AMELOG.**	X	Y	X	X	X	Y

Table 4.11:Shows The 16 Core Set of STR Loci
Case No.9 (Paternity)

No	Genetic Loci	9A:Alleged Father		9B : Mother		9C : Child	
		Allele1	Allele2	Allele1	Allele2	Allele1	Allele2
1	**D8S1179**	13	16	11	15	11	16
2	**D21S11**	30	32.2	30	33.2	30	32.2
3	**D7S820**	10	10	9	10	9	10
4	**CSF1PO**	11	14	8	9	8	11
5	**D3S1358**	15	17	17	17	17	17
6	**TH01**	7	8	6	9.3	7	9.3
7	**D13S317**	11	12	9	11	9	12
8	**D16S539**	9	14	9	9	9	14
9	**D2S1338**	19	20	17	20	17	20
10	**D19S433**	13	14	12	14	13	14
11	**VWA**	15	17	16	17	15	17
12	**TPOX**	9	11	11	11	9	11
13	**D18S51**	15	17	12	14	14	17
14	**D5S818**	12	12	10	12	12	12
15	**FGA**	25	29	23	28	25	28
16	**AMELOG.**	X	Y	X	X	X	Y

Table 4.12:Shows The 16 Core Set of STR Loci Case No.10 (Paternity)

NO	Genetic Loci	10A:Alleged Father		10B : Mother		10C : Child	
		Allele1	Allele2	Allele1	Allele2	Allele1	Allele2
1	**D8S1179**	11	11	13	14	14	16*
2	**D21S11**	30	31.2	28	35	28	32*
3	**D7S820**	9	9	8	10	8*	10
4	**CSF1PO**	10	13	8	12	11*	12
5	**D3S1358**	15	17	16	17	15	17
6	**TH01**	7	9	6	7	7	7
7	**D13S317**	11	14	11	12	12*	12
8	**D16S539**	11	11	10	11	11	12*
9	**D2S1338**	22	23	22	22	20*	22
10	**D19S433**	12	14	13.2	15	14	15
11	**VWA**	16	17	14	18	16	18
12	**TPOX**	8	11	9	9	9*	9
13	**D18S51**	11	13	17	18	17*	18
14	**D5S818**	11	12	11	12	12	12
15	**FGA**	26	28	22	23	22*	23
16	**AMELOG.**	X	Y	X	X	X	Y

*refer to non matching allele

Table 4.13:Shows The 16 Core Set of STR Loci Case No.11 (Paternity)

No	Genetic Loci	11A:Alleged Father		11B : Mother		11C : Child	
		Allele1	Allele2	Allele1	Allele2	Allele1	Allele2
1	**D8S1179**	13	14	14	14	14	14
2	**D21S11**	30	32.2	29	30	30	30
3	**D7S820**	9	10	11	11	9	11
4	**CSF1PO**	10	12	10	11	10	10
5	**D3S1358**	16	17	17	18	16	18
6	**TH01**	7	8	9	9	7	9
7	**D13S317**	12	13	12	12	12	13
8	**D16S539**	13	13	11	13	11	13
9	**D2S1338**	18	18	20	22	18	20
10	**D19S433**	13	14	14	14	14	14
11	**VWA**	13	20	15	16	13	16
12	**TPOX**	8	8	6	8	8	8
13	**D18S51**	14	16	15	17	14	17
14	**D5S818**	12	12	12	13	12	12
15	**FGA**	21	22	19	23	19	21
16	**AMELOG.**	**X**	**Y**	**X**	**X**	**X**	**X**

Table 4.14:Shows The 16 Core Set of STR Loci
Case No.12 (Paternity)

NO	Genetic Loci	12A:Alleged Father		12B : Mother		12C : Child	
		Allele1	Allele2	Allele1	Allele2	Allele1	Allele2
1	**D8S1179**	11	15	12	13	13	15
2	**D21S11**	28	30	28	29	28	29
3	**D7S820**	11	12	9	10	9	11
4	**CSF1PO**	10	11	9	12	9	11
5	**D3S1358**	15	17	15	16	15	17
6	**TH01**	10	10	6	7	6	10
7	**D13S317**	12	12	12	12	12	12
8	**D16S539**	9	12	9	12	9	12
9	**D2S1338**	17	20	19	21	19	20
10	**D19S433**	13	16	13.2	14.2	13	13.2
11	**VWA**	15	16	16	19	16	19
12	**TPOX**	8	9	8	11	8	8
13	**D18S51**	17	17	17	20	17	20
14	**D5S818**	11	13	12	12	12	13
15	**FGA**	22	24	28	30	22	28
16	**AMELOG.**	**X**	**Y**	**X**	**X**	**X**	**X**

Table 4.15:Shows The 16 Core Set of STR Loci
Case No.13 (Paternity)

*refer to non matching allele

NO	Genetic Loci	13A:Alleged Father		13B : Mother		13C : Child	
		Allele1	Allele2	Allele1	Allele2	Allele1	Allele2
1	**D8S1179**	12	13	14	14	14*	14
2	**D21S11**	28	31.2	28	29	28	28
3	**D7S820**	10	11	10	10	9*	10
4	**CSF1PO**	10	11	12	12	12*	12
5	**D3S1358**	16	17	15	18	17	18
6	**TH01**	7	7	6	6	6*	6
7	**D13S317**	11	13	8	11	8	13
8	**D16S539**	11	13	11	12	9*	11
9	**D2S1338**	23	26	19	22	18*	19
10	**D19S433**	14	14	14	14	14	16.2*
11	**VWA**	15	16	17	20	17	18*
12	**TPOX**	8	8	9	9	9	10*
13	**D18S51**	13	14	12	18	18	19*
14	**D5S818**	12	12	12	13	13*	13
15	**FGA**	21	23	21	21	21	21
16	**AMELOG.**	**X**	**Y**	**X**	**X**	**X**	**X**

Table 4.16:Shows The 16 Core Set of STR Loci
Case No.14 (Paternity)

NO	Genetic Loci	14A:Alleged Father		14B : Mother		14C : Child 1		14D : Child 2	
		Allele1	Allele2	Allele1	Allele2	Allele1	Allele2	Allele1	Allele2
1	**D8S1179**	13	14	12	14	12	14	12	14
2	**D21S11**	29	30	28	29	29	30	29	29
3	**D7S820**	8	9	8	8	8	9	8	9
4	**CSF1PO**	9	11	12	13	9	12	9	13
5	**D3S1358**	14	15	14	16	15	16	14	14
6	**TH01**	7	9	6	7	7	9	6	9
7	**D13S317**	8	11	12	13	11	12	11	13
8	**D16S539**	9	12	9	13	12	13	9	13
9	**D2S1338**	18	24	22	24	24	24	22	24
10	**D19S433**	14	15.2	14	14	14	15.2	14	15.2
11	**VWA**	16	17	15	16	15	16	16	17
12	**TPOX**	8	9	9	9	8	9	9	9
13	**D18S51**	14	16	15	16	14	16	14	15
14	**D5S818**	12	13	12	13	12	13	12	13
15	**FGA**	23	25	23	23	23	25	23	25
16	**AMELOG.**	**X**	**Y**	**X**	**X**	**X**	**Y**	**X**	**X**

Table 4.17:Shows The 16 Core Set of STR Loci
Case No.15 (Paternity)

NO	Genetic Loci	15A:Alleged father1		15B:Alleged father2		15C:Alleged father3		15D : Mother		15F: Child	
		Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
1	**D8S1179**	13	16	13	15	10	13	14	15	14	15
2	**D21S11**	29	30	29	30	29	30	30	31	30	30
3	**D7S820**	9	10	9	10	10	10	8	10	10	10
4	**CSF1PO**	12	12	10	10	9	12	10	13	10	13
5	**D3S1358**	17	18	15	16	15	16	16	18	16	16
6	**TH01**	8	9	6	7	6	8	7	8	6	8
7	**D13S317**	11	11	11	12	12	12	11	14	11	11
8	**D16S539**	12	13	11	11	12	14	11	12	11	12
9	**D2S1338**	20	20	24	24	18	25	20	22	20	24
10	**D19S433**	12	14	13.2	14	13	15	13	13.2	13.2	13.2
11	**VWA**	16	17	17	17	18	18	14	18	14	17
12	**TPOX**	8	11	8	11	8	8	9	9	9	11
13	**D18S51**	13	15	12	19	12	14	16	20	12	16
14	**D5S818**	8	11	9	12	12	12	9	12	12	12
15	**FGA**	19	20	19	28	21	27	22	23	19	22
16	**AMELOG.**	**X**	**Y**	**X**	**Y**	**X**	**Y**	**X**	**X**	**X**	**X**

Table 4.18:Shows The 16 Core Set of STR Loci
Case No.16 (Rape)

NO	Genetic Loci	16A: semen stain (victim's clothes)		16B: FTA card (suspect)		16C: Sheet	
		Allele1	Allele2	Allele1	Allele2	Allele1	Allele2
1	**D8S1179**	12	15	12	15	12	15
2	**D21S11**	29	31.2	29	31.2	29	31.2
3	**D7S820**	8	8	8	-8	8	8
4	**CSF1PO**	10	12	10	12	10	12
5	**D3S1358**	15	15	15	15	15	15
6	**TH01**	6	9.3	6	9.3	6	9.3
7	**D13S317**	11	11	11	11	11	11
8	**D16S539**	12	12	12	12	12	12
9	**PENTA E**	10	16	10	16	10	16
10	**PENTA D**	10	14	10	14	10	14
11	**VWA**	16	17	16	17	16	17
12	**TPOX**	8	10	8	10	8	10
13	**D18S51**	18	22	18	22	18	22
14	**D5S818**	12	12	12	12	12	12
15	**FGA**	20	24	20	24	20	24
16	**AMELOG.**	**X**	**Y**	**X**	**Y**	**X**	**Y**

Table 4.19:Shows The 16 Core Set of STR Loci Case No.17 (Rape)

NO	Genetic loci	17A : child's clothes		17B: Suspect's clothes	
		Allele1	Allele2	Allele1	Allele2
1	**D8S1179**	**13**	**15**	**13**	**15**
2	**D21S11**	**29**	**29**	**29**	**29**
3	**D7S820**	**9**	**11**	**9**	**11**
4	**CSF1PO**	**10**	**12**	**10**	**12**
5	**D3S1358**	**16**	**16**	**16**	**16**
6	**TH01**	**9.3**	**10**	**9.3**	**10**
7	**D13S317**	**12**	**12**	**12**	**12**
8	**D16S539**	**8**	**9**	**8**	**9**
9	**PENTA E**	**5**	**8**	**5**	**8**
10	**PENTA D**	**2.2**	**12**	**2.2**	**12**
11	**VWA**	**16**	**19**	**16**	**19**
12	**TPOX**	**8**	**8**	**8**	**8**
13	**D18S51**	**17**	**19**	**17**	**19**
14	**D5S818**	**8**	**11**	**8**	**11**
15	**FGA**	**20**	**21**	**20**	**21**
16	**AMELOG.**	**X**	**Y**	**X**	**Y**

Table 4.20:Shows The 16 Core Set of STR Loci Case NO.18 (Rape)

NO	Genetic loci	18A: victim 's cloth		18B: FTA card Suspect 's Blood	
		Allele1	Allele2	Allele1	Allele2
1	**D8S1179**	**13**	**15**	**13**	**14**
2	**D21S11**	**29**	**31.2**	**28**	**31**
3	**D7S820**	**7**	**7**	**8**	**10**
4	**CSF1PO**	**10**	**10**	**10**	**10**
5	**D3S1358**	**15**	**15**	**16**	**17**
6	**TH01**	**6**	**9.3**	**7**	**8**
7	**D13S317**	**8**	**11**	**11**	**13**
8	**D16S539**	**11**	**12**	**9**	**12**
9	**PENTA E**	**11**	**16**	**16**	**16**
10	**PENTA D**	**10**	**14**	**11**	**11**
11	**VWA**	**16**	**22**	**16**	**16**
12	**TPOX**	**8**	**10**	**11**	**11**
13	**D18S51**	**18**	**22**	**18**	**19**
14	**D5S818**	**12**	**12**	**8**	**12**
15	**FGA**	**20**	**24**	**22**	**24**
16	**AMELOG.**	**X**	**Y**	**X**	**X**

Table 4.21:Shows The 16 Core Set of STR Loci
Case No.19 (Rape)

NO	Genetic Loci	19A:swab (victim)		19B:liquid blood (suspect)		19C: cloth (victim)	
		Allele1	Allele2	Allele1	Allele2	Allele1	Allele2
1	**D8S1179**	13	14	14	16	14	16
2	**D21S11**	30	33.2	29	29	29	29
3	**D7S820**	10	12	8	10	8	10
4	**CSF1PO**	10	11	9	12	9	12
5	**D3S1358**	15	16	16	17	16	17
6	**TH01**	6	7	7	7	7	7
7	**D13S317**	8	11	11	11	11	11
8	**D16S539**	9	11	10	11	10	11
9	**D2S1338**	18	18	17	19	17	19
10	**D19S433**	12.2	14	14	14	14	14
11	**VWA**	14	16	14	17	14	17
12	**TPOX**	8	10	8	11	8	11
13	**D18S51**	16	16	12	18.2	12	18.2
14	**D5S818**	12	15	11	12	11	12
15	**FGA**	23	28	25	28	25	28
16	**AMELOG.**	**X**	**Y**	**X**	**Y**	**X**	**Y**

Table 4.22:Shows The 16 Core Set of STR Loci
Case No.20 (Rape)

NO	Genetic Loci	20A:swab (victim)		20B:liquid blood (suspect)		20C: clothes (victim)	
		Allele1	Allele2	Allele1	Allele2	Allele1	Allele2
1	D8S1179	12	12	13	14	13	14
2	D21S11	28	29	32.2	34	32.2	34
3	D7S820	8	8	10	11	10	11
4	CSF1PO	11	11	10	12	10	12
5	D3S1358	16	16	14	15	14	15
6	TH01	7	7	6	7	6	7
7	D13S317	11	12	9	9	9	9
8	D16S539	11	13	9	9	9	9
9	D2S1338	20	25	22	25	22	25
10	D19S433	13.2	14	12.2	13	12.2	13
11	VWA	15	17	16	17	16	17
12	TPOX	8	8	8	9	8	9
13	D18S51	16	17	14	20	14	20
14	D5S818	11	13	13	13	13	13
15	FGA	19	2	22	23	22	23
16	AMELOG.	X	Y	X	Y	X	Y

Table 4.23:Shows The 16 Core Set of STR Loci Case No.21 (Rape)

NO	Genetic Loci	21A: clothes (victim)		21B:liquid blood (suspect)	
		Allele1	Allele2	Allele1	Allele2
1	**D8S1179**	12	14	13	14
2	**D21S11**	29	30	29	31.2
3	**D7S820**	10	10	10	12
4	**CSF1PO**	12	13	12	14
5	**D3S1358**	15	18	16	18
6	**TH01**	6	7	6	9
7	**D13S317**	12	12	11	13
8	**D16S539**	8	12	9	11
9	**D2S1338**	21	23	19	20
10	**D19S433**	13	14	14	14
11	**VWA**	16	17	16	17
12	**TPOX**	9	10	8	8
13	**D18S51**	17	19	15	15
14	**D5S818**	8	11	12	13
15	**FGA**	23	23	21	25
16	**AMELOG.**	X	Y	X	Y

Table 4.24:Shows The 16 Core Set of STR Loci
Case No.22 (Rape)

NO	Genetic L	22A liquid blood (victim)		22B liquid blood (suspect 1)		22C liquid blood (suspect 2)		22DLiquid sample (victim)	
		Allele1	Allele2	Allele1	Allele2	Allele1	Allele2	Allele1	Allele2
1	**D8S1179**	13	14	13	15	11	15	13	14
2	**D21S11**	28	31.2	30	35	29	29	28	31.2
3	**D7S820**	9	11	12	12	8	11	9	11
4	**CSF1PO**	11	11	8	12	7	11	11	11
5	**D3S1358**	15	16	15	15	15	17	15	16
6	**TH01**	6	9.3	7	10	7	7	6	9.3
7	**D13S317**	11	14	12	14	8	11	11	14
8	**D16S539**	11	14	11	11	9	9	11	14
9	**D2S1338**	17	20	20	20	22	22	17	20
10	**D19S433**	13.2	14.2	13	13	15	15	13.2	14.2
11	**VWA**	15	16	15	18	17	17	15	16
12	**TPOX**	8	9	8	11	8	11	8	9
13	**D18S51**	11	14	10.2	15	16	17	11	14
14	**D5S818**	9	12	12	13	11	13	9	12
15	**FGA**	23	23	20	22	22	25	23	23
16	**AMELO G.**	X	X	X	Y	X	Y	X	X

Table 4.25:Shows The 16 Core Set of STR Loci
Case No.23 (Rape)

NO	Genetic Loci	23A:liquid blo (victim)		23B:liquid blood (suspect)		23C:swab (victim)		23D:clothes (victim)
		Allele1	Allele2	Allele1	Allele2	Allele1	Allele2	Mix*
1	**D8S1179**	13	14	14	14	13	14	13,14
2	**D21S11**	29	32.2	29	36	29	32.2	29,32.2,36
3	**D7S820**	9	9	8	10	9	9	8,9,10
4	**CSF1PO**	8	9	12	12	8	9	8,9,12
5	**D3S1358**	16	16	15	16	16	16	15,16
6	**TH01**	7	9	7	7	7	9	7,9
7	**D13S317**	12	13	11	12	12	13	11,12,13
8	**D16S539**	11	12	11	11	11	12	11,12
9	**D2S1338**	19	22	18	22	19	22	18,19,22
10	**D19S433**	14	14.2	13	14	14	14.2	13,14,14.2
11	**VWA**	15	18	16	17	15	18	15,16,17,18
12	**TPOX**	8	10	9	13	8	10	8,9,10,13
13	**D18S51**	15	20	18	20	15	20	15,18,20
14	**D5S818**	12	13	11	13	12	13	11,12,13
15	**FGA**	23	28	22	24	23	28	22,23,24,28
16	**AMELO G.**	X	X	X	Y	X	X	X,Y

*mixed stain which were consistent with both male and female material left on the victim's clothes

Table 4.26:Shows The 16 Core Set of STR Loci Case. No .24 (Rape)

NO	Genetic Loci	24A:liquid blood (suspect)		24B:liquid blood (victim)		24C1:swab (victim)	24C2:swab) (victim)	
		Allele1	Allele2	Allele 1	Allele2	Mix*	Allele1	Allele 2
1	**D8S1179**	14	14	14	14	14	14	14
2	**D21S11**	29	30	28	29	28 , 29,30	29	30
3	**D7S820**	7	11	8	10	7 , 8,10 , 11	7	11
4	**CSF1PO**	11	12	8	11	8 , 11,12	11	12
5	**D3S1358**	16	17	15	16	15 , 16,17	16	17
6	**TH01**	7	10	7	8	7 , 8,10	7	10
7	**D13S317**	12	12	12	12	12	12	12
8	**D16S539**	12	13	9	12	9 , 12,13	12	13
9	**D2S1338**	18	20	18	24	18 , 20,24	18	20
10	**D19S433**	13	13.2	13	15.2	13 , 13.2,15.2	13	13.2
11	**VWA**	16	17	14	17	14 , 16,17	16	17
12	**TPOX**	9	10	8	9	8 , 9,10	9	10
13	**D18S51**	15	18	17	21	15 , 17,18 , 21	15	18
14	**D5S818**	12	12	10	13	10 , 12,13	12	12
15	**FGA**	20	23	25	27	20 , 23,25 , 27	20	23
16	**AMELOG.**	X	Y	X	X	XY	X	Y

*mixed stain which were consistent with both male and female material left on the victim body.

Table 4.27:Shows The 16 Core Set of STR Loci Case No. 25 (Rape)

NO	Genetic Loci	25A:liquid blood (suspect)		25B:liquid blood (victim)		25C blood stain (victim's cloth
		Allele1	Allele2	Allele1	Allele2	Mix*
1	**D8S1179**	13	13	12	17	12 , 13,17
2	**D21S11**	28	29	28	32.2	28 , 29,32.2
3	**D7S820**	11	11	10	10	10,11
4	**CSF1PO**	10	10	11	12	10,11 ,12
5	**D3S1358**	16	17	15	16	15 , 16,17
6	**TH01**	6	8	6	9.3	6 , 8,9.3
7	**D13S317**	11	12	11	13	11,12 ,13
8	**D16S539**	12	12	9	9	9,12
9	**D2S1338**	20	23	18	21	18 , 20,21 , 23
10	**D19S433**	15.2	17.2	14	14	14 , 15.2,17.2
11	**VWA**	17	18	16	16	16 , 17,18
12	**TPOX**	8	9	9	9	8,9
13	**D18S51**	12	16	15	18	12 , 15,16
14	**D5S818**	10	13	12	12	10 , 12,13
15	**FGA**	21	23	19	21	19 , 21,23
16	**AMELOG.**	X	Y	X	X	XY

*mixed stain which were consistent with both male and female material left on the victim's clothes

Table 4.28:Shows The 16 Core Set of STR Loci Case No. 26 (Murder)

No	Genetic loci	26A: blood swab (victim)		26B: blood stain (clothes) (suspect)	
		Allele1	Allele2	Allele1	Allele2
1	**D8S1179**	13	13	13	13
2	**D21S11**	29	29	29	29
3	**D7S820**	10	11	10	11
4	**CSF1PO**	7	13	7	13
5	**D3S1358**	14	15	14	15
6	**TH01**	7	9	7	9
7	**D13S317**	10	11	10	11
8	**D16S539**	11	14	11	14
9	**PENTA E**	13	15	13	15
10	**PENTA D**	9	12	9	12
11	**VWA**	15	17	15	17
12	**TPOX**	8	10	8	10
13	**D18S51**	12	13	12	13
14	**D5S818**	8	13	8	13
15	**FGA**	21	27	21	27
16	**AMELOG.**	X	Y	X	Y

Table 4.29:Shows The 16 Core Set of STR Loci Case No. 27 (Murder)

No	Genetic loci	27A: blood stain (suspect's clothes)		27B: blood stain (victim's clothes)	
		Allele1	Allele2	Allele1	Allele2
1	**D8S1179**	12	14	13	13
2	**D21S11**	25	31	28	30
3	**D7S820**	8	12	10	11
4	**CSF1PO**	10	12	10	10
5	**D3S1358**	15	16	16	17
6	**TH01**	6	7	7	8
7	**D13S317**	12	12	11	12
8	**D16S539**	11	11	11	12
9	**PENTA E**	16	18	8	10
10	**PENTA D**	2.2	11	7	13
11	**VWA**	16	19	14	15
12	**TPOX**	7	9	8	8
13	**D18S51**	15	17	18	19
14	**D5S818**	8	12	8	11
15	**FGA**	19	25	21	24
16	**AMELOG.**	X	Y	X	Y

Table 4.30: Shows The 16 Core Set of STR Loci Case No. 28 (Murder)

NO	Genetic loci	28A: blood stain (suspect's clothes)		28B: blood stain (victim)	
		Allele1	Allele2	Allele1	Allele2
1	**D8S1179**	12	14	12	14
2	**D21S11**	28	32.2	32.2	32.2
3	**D7S820**	8	8	8	8
4	**CSF1PO**	11	11	11	11
5	**D3S1358**	15	17	15	17
6	**TH01**	7	9	7	9
7	**D13S317**	8	12	8	12
8	**D16S539**	12	12	11	12
9	**PENTA E**	7	8	7	8
10	**PENTA D**	8	8	8	8
11	**VWA**	15	16	15	16
12	**TPOX**	8	9	8	9
13	**D18S51**	14	24	14	18
14	**D5S818**	10	12	10	12
15	**FGA**	28	28	28	28
16	**AMELOG.**	X	Y	X	Y

Table 4.31: Shows The 16 Core Set of STR Loci Case No. 29 (Murder)

NO	Genetic loci	29A : blood stain (suspect's clothes)		29B: blood (victim)	
		Allele1	Allele2	Allele1	Allele2
1	**D8S1179**	12	14	12	14
2	**D21S11**	29	30	29	30
3	**D7S820**	11	12	11	12
4	**CSF1PO**	12	12	12	12
5	**D3S1358**	15	15	15	15
6	**TH01**	7	7	7	7
7	**D13S317**	8	12	8	12
8	**D16S539**	10	12	10	12
9	**PENTA E**	7	7	7	7
10	**PENTA D**	9	11	9	11
11	**VWA**	16	17	16	17
12	**TPOX**	8	9	8	9
13	**D18S51**	18	20	18	20
14	**D5S818**	11	13	11	13
15	**FGA**	21	24	21	24
16	**AMELOG.**	X	Y	X	Y

Table 4.32 :Shows The 16 Core Set of STR Loci Case No. 30(Murder)

NO	Genetic loci	30A: blood stain (suspect's clothes)		30B: blood stain (victim)	
		Allele1	Allele2	Allele1	Allele2
1	**D8S1179**	11	15	13	15
2	**D21S11**	29	32	29	32
3	**D7S820**	11	13	10	10
4	**CSF1PO**	10	12	8	11
5	**D3S1358**	13	15	15	15
6	**TH01**	6	7	7	9
7	**D13S317**	9	11	11	13
8	**D16S539**	10	12	9	12
9	**PENTA E**	15	16	7	7
10	**PENTA D**	10	10	2.2	7
11	**VWA**	15	15	16	18
12	**TPOX**	8	10	8	10
13	**D18S51**	16	21	16	16
14	**D5S818**	12	13	12	13
15	**FGA**	19	23	22	25
16	**AMELOG.**	X	Y	X	Y

Table 4.33:Shows The 16 Core Set of STR Loci Case No. 31 (Murder)

NO	Genetic Loci	31A:Blood Stain (Crime scene)		31B:blood stain suspect's clothes	31C: Liquid blood. (victim)		31D: Liquid blood (suspect)	
		Allele1	Allele2	Mix*	Allele1	Allele2	Allele1	Allele 2
1	**D8S1179**	13	13	13,14,16	13	13	13	16
2	**D21S11**	29	36	29,30,31,36	29	36	30	31
3	**D7S820**	9	11	9,11	9	11	11	11
4	**CSF1PO**	10	11	10,11	10	11	8	10
5	**D3S1358**	16	17	13,16,17	16	17	13	17
6	**TH01**	6	7	6,7,9	6	7	7	9
7	**D13S317**	11	13	8,11,12,13	11	13	8	12
8	**D16S539**	11	13	11,13	11	13	11	11
9	**D2S1338**	18	22	18,22	18	22	24	25
10	**D19S433**	13.2	15	13.2,14,15	13.2	15	14	14
11	**VWA**	16	16	15,16,17	16	16	15	17
12	**TPOX**	11	11	9,11	11	11	9	11
13	**D18S51**	17	18	16,17,18	17	18	16	17
14	**D5S818**	11	13	11,12,13	11	13	11	12
15	**FGA**	21	28	21,22,28	21	28	21	22
16	**AMELO G.**	X	Y	X,Y	X	Y	X	Y

*mixed stain which were consistent with both suspect's clothes and other stain material

Table 4.34:Shows The 16 Core Set of STR Loci
Case No. 32(Murder)

NO	Genetic Loci	32A: blood stain (Cotton) (suspect's car)		32B: blood stain (Cotton) (victim)	
		Allele1	Allele2	Allele1	Allele2
1	D8S1179	13	14	13	14
2	**D21S11**	28	29	28	29
3	**D7S820**	11	12	11	12
4	**CSF1PO**	9	12	9	12
5	**D3S1358**	15	15	15	15
6	**TH01**	7	8	7	8
7	**D13S317**	12	13	12	13
8	**D16S539**	9	9	9	9
9	**D2S1338**	19	20	19	20
10	**D19S433**	14.2	16	14.2	16
11	**VWA**	16	17	16	17
12	**TPOX**	9	9	9	9
13	**D18S51**	11	17	11	17
14	**D5S818**	11	12	11	12
15	**FGA**	23	23	23	23
16	**AMELOG.**	X	Y	X	Y

Table 4.35:Shows The 16 Core Set of STR Loci Case No. 33 (Murder)

NO	Genetic Loci	33A:liquid blood (victim)		33B :blood stain (suspect's knife)		33C:blood stain (suspect's clothes)	
		Allele1	Allele2	Allele1	Allele2	Allele1	Allele2
1	**D8S1179**	11	14	11	14	10	14
2	**D21S11**	28	29	28	29	28	30
3	**D7S820**	8	11	8	11	8	11
4	**CSF1PO**	9	10	9	10	8	8
5	**D3S1358**	16	16	16	16	15	15
6	**TH01**	8	9.3	8	9.3	7	7
7	**D13S317**	12	12	12	12	11	11
88	**D16S539**	9	9	9	9	9	12
9	**D2S1338**	22	22	22	22	19	22
10	**D19S433**	13	16.2	13	16.2	12	15
11	**VWA**	16	16	16	16	15	16
12	**TPOX**	8	10	8	10	9	12
13	**D18S51**	16	18	16	18	15	17
14	**D5S818**	11	12	11	12	12	13
15	**FGA**	22	27	22	27	20	30
16	**AMELOG.**	X	X	X	X	X	Y

Table 4.36:Shows The 16 Core Set of STR Loci

Case No. 34 (Murder)

Genetic Loci	34A: blood Stain.paper crime scene		34B: Shoes		34C: clothes (Suspect 1)		34D: Clothes (Suspect 2)		34E: liquid blo (Victim)		34F:Soil sampl (Crime scene)	
	Alle1le 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
D8S1179	13	14	15	16	12	13	14	16	13	14	13	14
D21S11	28	28	30	35	30	31	28	30	28	28	28	28
D7S820	8	8	10	11	9	10	10	10	8	8	8	8
CSF1PO	12	12	11	12	10	12	8	12	12	12	12	12
D3S1358	17	17	16	16	16	17	15	15	17	17	17	17
TH01	6	7	6	6	7	8	7	9. 3	6	7	6	7
D13S317	12	13	11	11	11	12	12	12	12	13	12	13
D16S539	12	14	11	13	9	11	9	13	12	14	12	14
D2S133 8	18	26	20	21	19	19	21	22	18	26	18	26
D19S433	12	14	11	13	12	13	12. 2	15	12	14	12	14
VWA	16	18	17	19	15	17	14	14	16	18	16	18
TPOX	8	11	8	11	8	9	8	8	8	11	8	11
D18S51	15	19	14	18	15	15	14	15	15	19	15	19
D5S818	12	13	12	13	12	12	12	12	12	13	12	13
FGA	21	23	22	23	24	24	23	24	21	23	21	23
AMELO G.	X	Y	X	Y	X	Y	X	Y	X	Y	X	Y

Table 4.37 :Shows The 16 Core Set of STR Loci

Case No. 35 (Murder)

NO	Genetic Loci	35 A: blood stain (swab) (victim)		35B: : blood stain (suspect's knife)	
		Allele1	Allele2	Allele1	Allele2
1	D8S1179	14	15	14	15
2	D21S11	30	31	30	31
3	D7S820	11	11	11	11
4	CSF1PO	12	13	12	13
5	D3S1358	15	17	15	17
6	TH01	6	8	6	8
7	D13S317	11	12	11	12
8	D16S539	10	14	10	14
9	D2S1338	18	20	18	20
10	D19S4	13	14	13	14
11	VWA	16	16	16	16
12	TPOX	8	11	8	11
13	D18S51	12	14	12	14
14	D5S818	10	10	10	10
15	FGA	20	22	20	22
16	AMELOG.	X	Y	X	Y

Table 4.38 :Shows The 16 Core Set of STR Loci Positive control*

NO	Genetic Loci	Allele 1	Allele 2
1	**D8S1179**	13	13
2	**D21S11**	30	30
3	**D7S820**	10	11
4	**CSF1PO**	10	12
5	**D3S1358**	14	15
6	**TH01**	8	9.3
7	**D13S317**	11	11
8	**D16S539**	11	12
9	**D2S1338**	19	23
10	**D19S433**	14	15
11	**VWA**	17	18
12	**TPOX**	8	8
13	**D18S51**	15	19
14	**D5S818**	11	11
15	**FGA**	23	24
16	**AMELOG.**	X	X

*One component of Applied Biosystems. AmpFlSTR® Identifiler™ Kit

Table 4.39 :Shows The 16 Core Set of STR Loci

Negative control*

NO	Genetic Loci	Allele	Allele
1	**D8S1179**	**-**	**-**
2	**D21S11**	**-**	**-**
3	**D7S820**	**-**	**-**
4	**CSF1PO**	**-**	**-**
5	**D3S1358**	**-**	**-**
6	**TH01**	**-**	**-**
7	**D13S317**	**-**	**-**
8	**D16S539**	**-**	**-**
9	**D2S1338**	**-**	**-**
10	**D19S433**	**-**	**-**
11	**VWA**	**-**	**-**
12	**TPOX**	**-**	**-**
13	**D18S51**	**-**	**-**
14	**D5S818**	**-**	**-**
15	**FGA**	**-**	**-**
16	**AMELOG.**	**-**	**-**

*One component of Applied Biosystems. AmpFlSTR® Identifiler™ Kit

Table 4.40:Shows The 16 Core Set of STR Loci the author DNA profile tested in Sudan

NO	Genetic Loci	Allele 1	Allele 2
1	**D8S1179**	14	15
2	**D21S11**	28	30
3	**D7S820**	8	10
4	**CSF1PO**	10	12
5	**D3S1358**	16	17
6	**TH01**	6	9
7	**D13S317**	11	12
8	**D16S539**	9	11
9	**D2S1338**	17	17
10	**D19S433**	13	14
11	**VWA**	14	15
12	**TPOX**	8	10
13	**D18S51**	15	15
14	**D5S818**	11	11
15	**FGA**	19	23
16	**AMELOG.**	X	X

Table4.41 :Shows The 16 Core Set of STR Loci the author DNA profile tested in Jordan

NO	Genetic Loci	Allele 1	Allele 2
1	**D8S1179**	14	15
2	**D21S11**	28	30
3	**D7S820**	8	10
4	**CSF1PO**	10	12
5	**D3S1358**	16	17
6	**TH01**	6	9
7	**D13S317**	11	12
8	**D16S539**	9	11
9	**PENTA E**	14	17
10	**PENTA D**	2.2	13
11	**VWA**	14	15
12	**TPOX**	8	10
13	**D18S51**	15	15
14	**D5S818**	11	11
15	**FGA**	19	23
16	**AMELOG.**	X	X

4.3 .Statistical Analysis:

The present study involved three types of criminal justice, namely paternity, rape and murder cases. For paternity, 15 cases were used, while for rape and murder 10 cases were used. Chi-square and Bayes,s test values were used to assess the DNA- based evidence as discrimination for criminal justice .

4.3.1. Classical test:

Pretest for murder collected sample was tested by using Benzidine and Antihuman kits and its result indicated that for both tests the results were +ve as shown in Table (4.1). Also seminal presumptive test was done by using Acid phosphate test, PSA Quick test kids and microscopic examination. The results of these tests revealed that test of Acid phosphate test and PSA Quick test kids resulted in +ve result for all rape cases, whereas for microscopic examination the test result of cases 3 (18A), 4 (19A), 5 (20A), 7 (21A) and 9 (23C) was –ve (absence of spermatozoa due to bacterial or fungal contamination) as shown in Table (4.2) .

4.3.2 .Molecular techniques by using ABI 310 Analyzer :

4.3.2.1. Paternity cases:

Table (4. 43) showed the DNA paternity test for the 15 cases of the defendant parents. As shown from the table that the cases 1, 2, 3, 4, 6, 8, 9, 11, 12, 14 and 15 had fathered the victim's child, whereas defendant cases 5, 7,10 and 13 were not. Case 5 did not match the DNA test of the victim child in loci 1, 3, 5, 6, 8, 9, 10 and 12, while case 7 did not match in loci 1, 2, 4, 6, 9, 12, 13, 14 and 15. On the other hand, case 10 was not match the DNA test of the victim child in loci 1, 2, 3, , 4, 7, 8, 9, 12, 13, and 15, whereas case 13 not matching the child test in loci 1, 3, 4, 6, 8, 9, 10, 11, 12, 13 and 14.

Data of Table (4.44) subjected to both Chi-square and Bayes test to test the null hypothesis (H_0) that for all studied cases p= ½ (0.5), which means that defendant is fathered the victim child, while the alternative hypothesis (H_a)stated that $P \neq ½$ (> 0.5), which mean that the defendant is not father of the victim child. When 2X2 contingency table (i.e. two categorical variables each with two categories) the chi-square tend to produce significance values that are too small (type1 error), therefore, Bayes suggested a correlation to the Person test:

Table 4.42: Pretest of blood and seminal presumptive by using different type of testing materials

Tested material	**Type of test**	**Test result**	
		+ve	**-ve**
Blood	Benzidine	16	0
	Antihuman	16	0
	Total	32	0
Seminal presumptive	Acid phosphate	16	0
	PSA quick	16	0
	Microscopic exam.	9	5*
	Total	41	5

Table 4.43: Contingency table showing the DNA paternity test result for 15 cases

No. of cases	Parents	DNA paternity test result		Total
		Matching	Not matching	
Case1	Father	16 (50.0)	0 (0.0)	16 (50.0)
	Mother	16 (50.0)	0 (0.0)	16 (50.0)
	Total	32 (100.0)	0 (0.0)	32 (100.0)
Case2	Father	16 (50.0)	0 (0.0)	16 (50.0)
	Mother	16 (50.0)	0 (0.0)	16 (50.0)
	Total	32 (100.0)	0 (0.0)	32 (100.0)
Case3	Father	16 (50.0)	0 (0.0)	16 (50.0)
	Mother	16 (50.0)	0 (0.0)	16 (50.0)
	Total	32 (100.0)	0 (0.0)	32 (100.0)
Case4	Father	16 (50.0)	0 (0.0)	16 (50.0)
	Mother	16 (50.0)	0 (0.0)	16 (50.0)
	Total	32 (100.0)	0 (0.0)	32 (100.0)
Case5	Father	9 (28.1)	7 (21.9)	16 (50.0)
	Mother	16 (50.0)	0 (0.0)	16 (50.0)
	Total	32 (100.0)	0 (0.0)	32 (100.0)
Case6	Father	16 (50.0)	0 (0.0)	16 (50.0)
	Mother	16 (50.0)	0 (0.0)	16 (50.0)
	Total	32 (100.0)	0 (0.0)	32 (100.0)
Case7	Father	9 (28.1)	7 (21.9)	16 (50.0)
	Mother	16 (50.0)	0 (0.0)	16 (50.0)
	Total	32 (100.0)	0 (0.0)	32 (100.0)
Case8	Father	16 (50.0)	0 (0.0)	16 (50.0)
	Mother	16 (50.0)	0 (0.0)	16 (50.0)
	Total	32 (100.0)	0 (0.0)	32 (100.0)
Case9	Father	16 (50.0)	0 (0.0)	16 (50.0)
	Mother	16 (50.0)	0 (0.0)	16 (50.0)
	Total	32 (100.0)	0 (0.0)	32 (100.0)
Case10	Father	10 (31.3)	6 (18.7)	16 (50.0)
	Mother	16 (50.0)	0 (0.0)	16 (50.0)
	Total	32 (100.0)	0 (0.0)	32 (100.0)
Case11	Father	16 (50.0)	0 (0.0)	16 (50.0)
	Mother	16 (50.0)	0 (0.0)	16 (50.0)
	Total	32 (100.0)	0 (0.0)	32 (100.0)
Case12	Father	16 (50.0)	0 (0.0)	16 (50.0)
	Mother	16 (50.0)	0 (0.0)	16 (50.0)
	Total	32 (100.0)	0 (0.0)	32 (100.0)
Case13	Father	11 (34.4)	5 (15.6)	16 (50.0)
	Mother	16 (50.0)	0 (0.0)	16 (50.0)
	Total	32 (100.0)	0 (0.0)	32 (100.0)
Case14	Father	16 (50.0)	0 (0.0)	16 (50.0)
	Mother	16 (50.0)	0 (0.0)	16 (50.0)
	Total	32 (100.0)	0 (0.0)	32 (100.0)
Case15	Father	16 (50.0)	0 (0.0)	16 (50.0)
	Mother	16 (50.0)	0 (0.0)	16 (50.0)
	Total	32 (100.0)	0 (0.0)	32 (100.0)

*: Values between parentheses represent the percentage of matching score from the tota

X^2(Chi-square) = Σ (observed – model)^2/ model

X^2(Chi-square) = Σ ((observed – model) – o.5)^2/ model

Bayes test is used in criminal trials as ultimately theorem relies on unproven rather than the known facts. It simplifies the combination of different kind of evidence, lab, test and clinical trials. Also it permits evaluation evidence in null hypothesis (H_0).

According to these statements, Table (4.44) and fig.10 demonstrate that father's16 loci for the 15 cases (16 X 15 = 240 loci) matching 41.9% (201) of the same loci of victim child, corresponding to 8.1% (39) of his loci did not matching. On the other hand, all mother loci were matching the child loci. Both Chi-square and Bayes calculated values as shown in the table (42.449 and 40.300, respectively) were statistically significant (> the tabulated at df = 1 = 3.84 at 5% and 6.63 at 1%), which means that the (H_0) should be rejected that not all father studied cases were ½. The P-value (0.000) was also < 0.01 which also indicating the significant situation.

4.3.2.2. Rape cases:

Table (4.45) shows that in all rape cases suspected tests were matched the victim test (as reference) for all 16 loci (100%), except cases 18 ,21,22 and 23 in which all suspected loci differed from victim loci, except locus 10 in case 3.For rape crime, the tested null hypothesis (H_0) is that P = 1 (100%), while (H_a) P ≠1. Table (4.46) shows that 80% (257) of suspected and victim loci cases from blood of different tools (cloths and swab) were matching, corresponding to 19.7% (63) not matching and the difference was statistically significant (P< 0.01) and accordingly (H_0) is rejected, which means that the expected typical loci for all studied cases was equal (100%), but some suspected were excluded.

4.3.2.3. Murder cases:

Table (4.47) shows that for suspect murder, blood tests (cloths, shoes or knife) of cases 26, 29, 31, 32, , 33 and 35 was matching the victim blood, while cases 27,28,30 and 24 did not. Table (4.48) indicates that out of 10 suspected murder cases with 16 loci, 80% (256) were matching the victim blood sample, while 20% (64) did not. Statistical analysis indicated that the difference was statistically significant (P< 0.01).

Table 4.44: Chi-square test showing the result of DNA paternity test for the 15 cases

Parents	DNA paternity test result		Total
	Matching	Not matching	
Father	201 (41.9)	39 (8.1)	240 (50.0)
Mother	240 (100.0)	0 (0.0)	240 (50.0)
Total	32 (100.0)	0 (0.0)	480 (100.0)
Chi-square	42.449		
Bayes cotinuity	40.300		
P - value	0.000		
Sig. level	**		

**: Significant difference at 1%

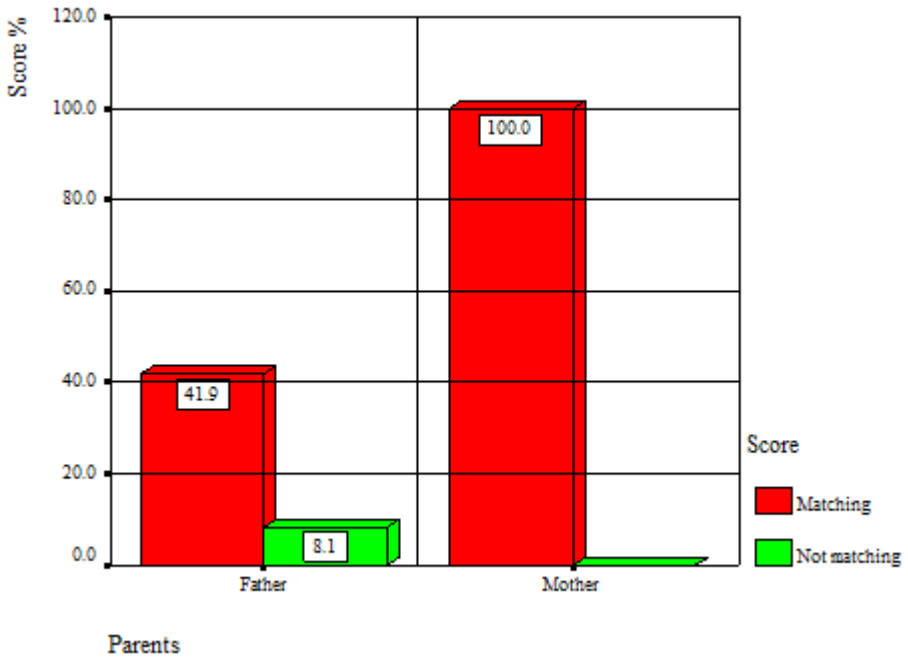


Figure 4.8: DNA test for paternity cases.

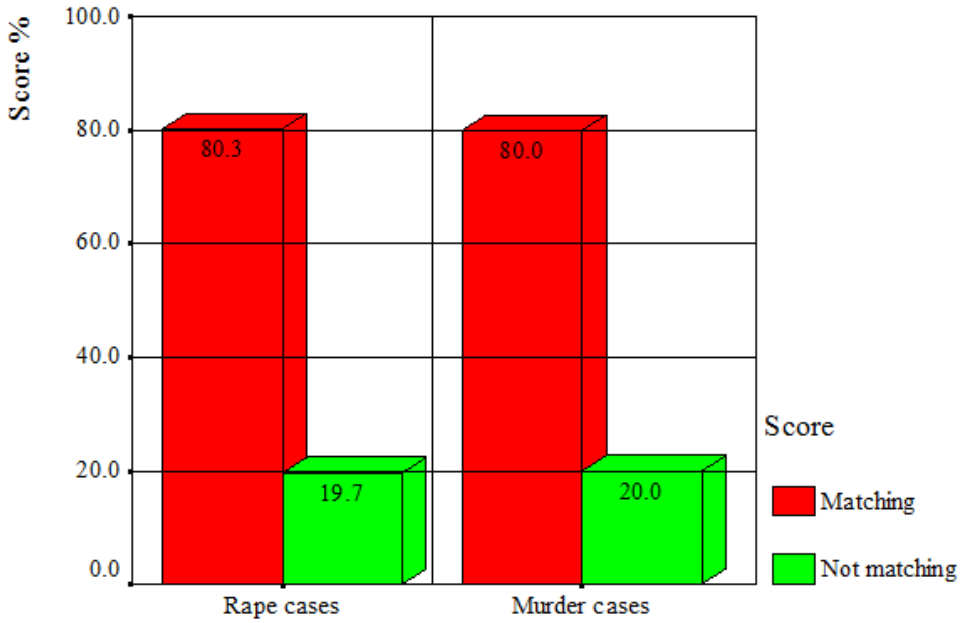
Table 4.45: Contingency table showing the rape test result for 10 cases

No. of cases	Parents	Rape test result		Total
		Matching	Not matching	
Case16	Suspect	16	0	16
	Victim	16	0	16
	Total	32	0	32
Case17	Suspect	16	0	16
	Victim	16	0	16
	Total	32	0	32
Case18	Suspect	1	15	16
	Victim	16	0	16
	Total	32	0	32
Case19	Suspect	16	0	16
	Victim	16	0	16
	Total	32	0	32
Case20	Suspect	16	0	16
	Victim	16	0	16
	Total	32	0	32
Case21	Suspect	0	16	16
	Victim	16	0	16
	Total	32	0	32
Case22	Suspect	0	16	16
	Victim	16	0	16
	Total	32	0	32
Cas23	Suspect	0	16	16
	Victim	16	0	16
	Total	32	0	32
Case24	Suspect	16	0	16
	Victim	16	0	16
	Total	32	0	32
Case25	Suspect	16	0	16
	Victim	16	0	16
	Total	32	0	32

Table 4.46: Chi-square test showing matching of 16 loci of 10 suspected rape cases.

Test result	Frequency	Percentage
Matching	257	83.3
Not matching	63	19.7
Total	320	100
Chi-square	58.286	
P - value	0.000	
Sig. level	**	

**: Significant difference at 1%



Cases

Figure 4.9: DNA test for rape and murder cases

Table 4.47: Contingency table showing the murder test result for 10 cases

No. of cases	Parents	Murder test result		Total
		Matching	Not matching	
Cas26	Suspect	16	0	16
	Victim	16	0	16
	Total	32	0	32
Case27	Suspect	0	16	16
	Victim	16	0	16
	Total	32	0	32
Cas28	Suspect	13	3	16
	Victim	16	0	16
	Total	32	0	32
Case29	Suspect	16	0	16
	Victim	16	0	16
	Total	32	0	32
Case30	Suspect	3	13	16
	Victim	16	0	16
	Total	32	0	32
Case31	Suspect	O	16	16
	Victim	16	0	16
	Total	32	0	32
Case32	Suspect	16	0	16
	Victim	16	0	16
	Total	32	0	32
Cas33	Suspect	16	0	16
	Victim	16	0	16
	Total	32	16	32
Case34	Suspect	0	0	16
	Victim	16	0	16
	Total	32	0	32
Case35	Suspect	16	0	16
	Victim	16	0	16
	Total	32	0	32

Table 4.48: Chi-square test showing matching of 16 loci of 10 suspected murder cases.

Test result	Frequency	Percentage
Matching	256	80
Not matching	64	20
Total	320	100
Chi-square	59.215	
P - value	0.000	
Sig. level	**	

**: Significant difference at 1%

Discussion

There are many methods available for extracting DNA e.g. organic extraction, Chelex extraction and by using Maxwell™ 16 automated DNA purification instrument ...etc. The choice of method to use depends on a number of factors, including the sample type and quantity.

In this study, there were 35 different cases collected from inside and outside Khartoum state, and their DNA were extracted, amplified and analyzed for the 16 loci. These loci are, D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S53, PENTA E, PENTA D, VWA, TPOX, D18S51, D5S818, FGA, AMELOG (Promega kits, Applied Bio system kits D2S1338, D19S433 instead of PENTA E and PENTA D respectively). DNA was extracted by different methods. It became clear that the chelex method is more rapid than phenol- chloroform- base extraction method (organic method). In addition, chelex extraction involve fewer steps and thus opportunities for sample – to – sample contamination. Whereas organic method could be considered less harsh than chelex beads because no boiling step is required. These two methods were used to extract DNA from samples collected from paternity and murder cases. The Boston Police Department crime laboratory currently has chelex 100 resin DNA extraction of whole blood, blood stain and hair. The only extraction method online for oral swab is the EZ I extraction method, but the best of them is extraction of DNA by using Maxwell™ 16 automated DNA purification instrument, the instrument can process up to 16 sample in 30 minutes.

Differential extraction method was used to isolate female and male fractions in sexual assault cases that contain a mixture of male and female DNA. By separating the male fraction away from the victim's DNA profile, it is much easier to interpret the perpetrator's DNA profile in rape cases. This method is conforms to the FBI laboratory and other forensic crime laboratories, while Promega corporation (Madison, WI) has developed an automated Differix method that involves using DNA IQ magnetic beads to hold the sperm pellet in place while a separation solution keeps the digestion buffer and epithelial DNA away from sperm pellet during the wash steps (Butler, 2012) .

The present study involve three types of criminal justice, namely paternity, rape and murder. Fifteen paternity cases, contained 17 samples had been taken from the alleged father to identify the original father of the child in dispute. These cases involve the mother, the child and one or more alleged father(s). The paternity testing is based on matches of the alleles at the 15 STR loci between the child, the mother and the alleged father. The DNA profiles of the child and the alleged father were matched in cases number 1, 2, 3, 4, 6, 8, 9, 11, 12, 14, and 15, that means the above alleged fathers were the biological father as shown in (tables 4.3 to 4.17). The test was considered a non – excluded paternity case. The DNA profiles of the child and alleged father were not matched in cases number 5, 7, 10, and 13. The result was considered an excluded paternity. Case 5, the child's DNA profile did not match in loci 1, 3, 5, 6, 8, 9, 10 and 12, while case 7 did not match in loci 1, 2, 4, 6, 9, 12, 13, 14 and 15. On the other hand, case 10 was not match the child's DNA profile in loci 1, 2, 3, , 4, 7, 8, 9, 12, 13, and 15, and also case 13 did not match the child's DNA profile in loci 1, 3, 4, 6, 8, 9, 10, 11, 12, 13 and 14 (tables 4.7, 4.9,4.12, 4.15), that means the above alleged fathers were

not the biological father. From the 15 cases studied only 11 cases matched the biological father (73.3%) which match the child's DNA. Father's loci for the 11 cases (16 X 15 = 240 loci) were matched 73.3% (176) of the same loci of child, corresponding to 26.7% (64) of his loci did not matched. On the other hand, all mother loci were matching the child's loci.

Ten sexual assault cases (tables 4.18 to 4.27) were also analyzed using different extraction methods for different evidences (suspect's clothes, blood taken from suspect), sheet, viginal swab from victim, victim's clothes, and blood taken from victim, for comparing the genotype pattern of the samples to identify the rapist. Five cases (case 16 ,case 17, case 19B, case 20B, and sample C2 in case 24) complete profile four 16 loci from suspect's DNA were matched with victim's DNA (inclusion), four cases (case 18, case 21, case 22 and case 23c) were not matched with victim's DNA (exclusion). Whereas samples 18A, 19A, 20A, 21 A and 22D spermatozoa did not appear (table 4.2). Case 23D and Case 25 were contaminated and gave mixed results. Sample C1 in case 24 was extracted by phenol-chloroform- base extraction method, gave mixed result due to mixed of sperms and epithelial cell from male and victim's female material left on the victim's body. The same sample was extracted by using differential extraction method gave complete profile matched with suspect's DNA(table 4.26). Therefore; male DNA extraction obtained with the differential method is the best method to be used in sexual crimes.

Different extraction methods were used in 10 murder cases (tables 4.28 to 4.37) the victim's DNA profile was compared to some evidences collected from the crime scene (suspect's clothes, suspect's car, suspect's knife) or blood taken from suspect, which led to identify the criminal, and link between victim's DNA on object in suspect's

possession. Cases number 26, 28, 29, 32, 33 and 35, complete victim profile were matched with suspect's possession, while cases 27,30, 31 and 24 did not. Victim's loci for 6 cases were matched (60%), while (40%) were not. The results suggested that there was no statistical difference between results obtained by different extraction methods investigated, but the Maxwell™ 16 automated DNA purification instrument robot made sample processing much simpler and quicker without introducing DNA contamination.

Conclusion

This study demonstrates that several DNA extraction methods have been developed and evaluated by forensic community. The selected method from biological samples influences the ability to successfully perform DNA analysis. In sexual assaults, differential method used to separate spermatozoa from other cell types. Therefore; male DNA extraction obtained with the differential method is the best method to be used in sexual crimes.

Recommendations

This study recommends using DNA differential method as the sole method for DNA extraction in sexual crime cases in police department.

Appendix

Appendix: Table.1: Information on 13 STR markers used in the FBI's CODIS DNA data base and other STR markers contained in commercial kits (Butler 2015).

Locus name	Chromosomal location	Repeat motif	GenBank accession	Allele range[a)]	Number of alleles seen[b)]
CSF1PO	5q33.1 c-fms proto-oncogene, 6th intron	TAGA	X14720	6–16	15
FGA	4q31.3 α-Fibrinogen, 3rd intron	CTTT	M64982	15–51.2	69
TH01	11p15.5 Tyrosine hydroxylase, 1st intron	TCAT	D00269	3–14	20
TPOX	2p25.3 Thyroid peroxidase, 10th intron	GAAT	M68651	6–13	10
VWA	12p13.31 von Willebrand factor, 40th intron	[TCTG][TCTA]	M25858	10–24	28
D3S1358	3q21.31	[TCTG][TCTA]	NT_005997	9–20	20
D5S818	5q23.2	AGAT	G08446	7–16	10
D7S820	7q21.11	GATA	G08616	6–15	22
D8S1179	8q24.13	[TCTA][TCTG]	G08710	8–19	13
D13S317	13q31.1	TATC	G09017	5–15	14
D16S539	16q24.1	GATA	G07925	5–15	10
D18S51	18q21.33	AGAA	L18333	7–27	43
D21S11	21q21.1	Complex [TCTA][TCTG]	AP000433	24–38	70
Other STRs included in kits from Applied Biosystems or Promega					
Penta D	21q22.3	AAAGA	AP001752	2.2–17	14 alleles
Penta E	15q26.2	AAAGA	AC027004	5–24	21 alleles
D2S1338	2q35	[TGCC][TTCC]	G08202	15–28	14 alleles
D19S433	19q12	AAGG	G08036	9–17.2	15 alleles
SE33	6q15	AAAG	V00481	4.2–37	>50 alleles

a. Numbers refer to the number of repeat units present in the alleles.
b. Numbers refer to the number of alleles.

Appendix: Table 2: commonly used STR kits on ABI 310 Analyzer

Table 2. Commonly used STR kits for analysis on ABI Prism 310 Genetic Analyzer

STR kit name	Source	Dye color	STR markers amplified in kit (shown in order of increasing PCR product size)
AmpFlSTR® Profiler Plus™	Applied Biosystems	B G Y	D3S1358, VWA, FGA Amelogenin, D8S1179, D21S11, D18S51 D5S818, D13S317, D7S820
AmpFlSTR COfiler™	Applied Biosystems	B G Y	D3S1358, D16S539 Amelogenin, TH01, TPOX, CSF1PO D7S820
AmpFlSTR SGM Plus™	Applied Biosystems	B G Y	D3S1358, VWA, D16S539, D2S1338 Amelogenin, D8S1179, D21S11, D18S51 D19S433, TH01, FGA
AmpFlSTR Identifiler™ (5-dyes)	Applied Biosystems	B G Y R	D8S1179, D21S11, D7S820, CSF1PO D3S1358, TH01, D13S317, D16S539, D2S1338 D19S433, VWA, TPOX, D18S51, Amelogenin, D5S818, FGA
AmpFlSTR SEfiler™ (5-dyes)	Applied Biosystems	B G Y R	D3S1358, VWA, D16S539, D2S1338 Amelogenin, D8S1179, SE33 D19S433, TH01, FGA D21S11, D18S51
PowerPlex® 1.2	Promega	B Y	D5S818, D13S317, D7S820, D16S539 VWA, TH01, Amelogenin, TPOX, CSF1PO
PowerPlex 16	Promega	B G Y	D3S1358, TH01, D21S11, D18S51, Penta E D5S818, D13S317, D7S820, D16S539, CSF1PO, Penta D Amelogenin, VWA, D8S1179, TPOX, FGA
PowerPlex ES	Promega	B G Y	D3S1358, TH01, D21S11, D18S51 SE33 (ACTBP2) Amelogenin, VWA, D8S1179, FGA
PowerPlex Y	Promega	B G Y	DYS391, DYS389I, DYS439, DYS389II DYS438, DYS437, DYS19, DYS392 DYS393, DYS390, DYS385 a/b
Y-PLEX™ 6	ReliaGene Technologies	B Y	DYS393, DYS19, DYS389II DYS390, DYS391, DYS385 a/b
Y-PLEX 5	ReliaGene Technologies	B G Y	DYS389I, DYS389II DYS439 DYS438, DYS392
Y-PLEX 12	ReliaGene Technologies	B G Y	DYS392, DYS390, DYS385 a/b DYS393, DYS389I, DYS391, DYS389II Amelogenin, DYS19, DYS439, DYS438

An internal size standard is typically run in the fourth or fifth dye position. Dye colors, blue (B), green (G), yellow (Y), or red (R).

DNA reagent preparation

1. **Benzidene solution:-**

one gram of benzidine powder was dissolved in 50 ml glacial acetic acid , then warmed to mix . The mixture was dissolved in 50 ml ethanol solution, and stored in brown bottle.

2. Acid phosphotase reagent :-

this reagent was prepared from two solutions (A) and (B) as follows:

solution (A) : 1gm of brantamine fast blue and 12gm of Sodium acetate were dissolved in 10 ml Glacial acetic acid . Mixture was completed to 100 ml distilled water.

Solution (B): 0.8 gm of Sodium α naphthyl phosphate were dissolved in 10 ml distilled water.

3. Eosin solution:-

One gram of Eosin powder was dissolved in 50 ml of distilled water.

4. Malakite green solution:-

One gram of Malakite powder was dissolved in 50 ml of distilled water.

5. Stain extraction buffer (100 ml):-

24.2gm of Tris base, 0.3738gm of Na_2- EDTA2H_2O, 0.584gm of NaCl and 0.602g of DTT were dissolved in 50 ml of distilled water pH was adjusted to 8.0 and 20 ml SDS added (10%), then completed to 100 ml d H_2O.

6. SDS (10%):-

10gm of SDS was dissolved in 70 ml of distilled water and heated To dissolve and completed to 100 ml distilled water.

7.1M DTT (10 ml):-

1.5425gm of DTT was dissolved in 10 ml of distilled water.

8. 0.5 EDTA, pH 8.0:-

186.1gm of $Na_2EDTA\text{-}2H_2O$ was dissolved in 700 ml of distilled water and then added 50 ml of 10N NaOH to pH 8.0.The mixture was Stirrered until dissolved, volume was brought to 1Liter with distilled water.

9. 5M NaCl:-

292.2g of NaCl was dissolved in 800ml of distilled water and adjust final volume to one liter, and autoclaved the solution .The solution was stored at room temperature

10. 10 mg/ml proteinase K:-

100 mg proteinase K was dissolved in 10 ml distilled or de-ionized water. The mixture was frozen and stored at -15 to -25 °C.

11. The TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0):-

10 ml of M Tris-HCl, pH 8.0, and 0.2 ml of 0.5 m EDTA were mixed together. The mixture was dissolved in 990 ml distilled or de-ionized water. The solution was autoclaved and stored at room temperature.

12. 1M Tris-HCl, pH 8.0:-

121.1g Tries base was dissolved in 800 ml distilled or deionizer water. pH was adjusted to 8.0 at room temperature by adding concentrated HCl (approximately 45 ml). The final volume was completed to 1 Litre with distilled or de-ionized water. The solution was autoclaved and stored at room temperature.

13. 0.5 M EDTA, pH 8.0:-

186.1gm of disodiumethylene-di-amine-tetra-acetate dehydrate ($Na_2EDTA\text{-}2\ H_2O$) was added To 800 ml distilled or de-ionized water.

14. Digest buffer:-

1ml of 1M Tris HCl was dissolved in 2ml of 0.5 M of EDTA, and then added 10 ml of 5 M NaCl, and 10 ml of 20% of SDS .The mixture was dissolved in 86 ml distilled or de-ionized water.

15. Lysis buffer:-

1ml of 1M HCl, pH 8.0 was dissolved in 2ml of 0.5 M EDTA, and then added 10 ml of 5 M NaCl

16. Buffered phenol-Chloroform-Isoamyl Alcohol solution:-

Mixed 25 parts purified phenol, 24 parts chloroform, and one part isoamylalcohol. Phenol-chloroform was Stored for up to 2 months at 2-6°C. Protect from light

17. Master Mix:-

1. the following reagents were vortexed for 5 seconds

-AmpfLSTR PCR Reaction mix.

-AmpliTag cold DNA polymerase.

-AmpfLSTR identifier primer set.

2. The required amount of components were calculated as shown:

1- Number of samples X 10.5 µl of AmpfLsTR PCR Reaction Mix.

2- Number of samples X 0.5 µl of AmpliTag Gold DNA polymerase.

3- Number of samples X 5.5 µl of AmpfLSTR identifier primer set the total number of samples were determined, including controls (positive and negative control).

3-The master mix was prepared by combining AmpfLSTR PCR Mix, AmpliTag Gold DNA polymerase, and AmpfLsTR identifier primer set Reagent:

18. The ABI 310 Genetic Analyzer reagents:-

1. The required amount of reagents was calculated as shown:
    1. Number of samples X2.5µHIDI formamide
    2. Number of samplesX0.5µL Gene Liz 500
    3. Placed it in one tube and vortexed to mix

(Number of samples including Allelic ladder).

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Glossary

Forensic DNA Testing Terminology

ABI 310 Genetic Analyzer – a capillary electrophoresis instrument used by forensic DNA laboratories to separate short tandem repeat (STR) loci on the basis of their size.

Adenine – a purine base; one of the four molecules containing nitrogen present in the nucleic acids DNA and RNA; designated by letter A.

Allele – one of two or more alternative forms of a gene.

Allele Frequency – the proportion of a particular allele among the chromosomes carried by individuals in a population.

ASCLD (ascld.org) – American Society of Crime Laboratory Directors; involved with accreditation of DNA testing labs.

Amino acid – Any of a class of 20 molecules that are combined to form proteins in living things . The sequence of amino acids in a protein and hence protein function are determined by the genetic code.

Amplification – An increase in the number of copies of a specific DNA fragment; can be in vivo or in vitro.

Autosome – A chromosome not involved in sex determination. The diploid human genome consists of 46 chromosomes, 22 pairs of autosomes, and one pair of sex chromosomes (the X and Y chromosomes).

Base pair – two complementary nucleotides in DNA; base pairing occurs between A and T and between G and C.

Base sequence – the order of nucleotide bases in a DNA molecule.

Base sequence analysis – a method, sometimes automated, for determining the base sequence.

Biotechnology – a set of biological techniques developed through basic research and now applied to research and product development.

Blind proficiency test – a proficiency test in which the laboratory personnel do not know that a test is being conducted.

Capillary electrophoresis – a method that utilizes a narrow polymer-filled tube to separate DNA molecules by size.

Ceiling principle – a conservative approach for estimating a DNA profile's frequency of occurrence in a population containing multiple ethnic groups.

Chromosome – a large piece of DNA. Humans have 23 different chromosomes in almost every type of cell.

CODIS – Combined DNA Index System, established in 1998 and containing the STR DNA profiles of many thousands of convicted offenders.

COfiler – PCR Amplification Kit (AmpFLSTR® COfiler™) that provide human identification laboratories with the ability to generate information for six STR loci and Amelogenin.

Complementary sequences – nucleic acid base sequences that form a double stranded structure by matching base pairs; the complementary sequence to G-T-AC is C-A-T-G.

Controls – tests performed in parallel with experimental samples and designed to demonstrate that a test was reliable.

Cytosine –pyrimidine base; one of the four molecules containing nitrogen present in the nucleic acids DNA and RNA; designated by letter C.

Degradation – the chemical or physical breaking down of DNA.

Denaturation – the process of splitting the complementary double strands of DNA to form single strands.

DNA (Deoxyribonucleic acid) – the genetic material.

Diploid – having two sets of chromosomes, one from each parent (compare haploid).

DNA databank (database) – a collection of DNA typing profiles of selected or randomly chosen individuals.

DNA polymerase – an enzyme that catalyzes the synthesis of double stranded DNA.

DNA sequence – the relative order of base pairs, whether in a fragment of DNA, a gene, a chromosome, or an entire genome.

Double Helix – the shape that two linear strands of DNA assume when bonded together.

Dye blobs – a technical artifact associated with STR testing.

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

Enzyme – a protein that can speed up a specific chemical reaction without being changed or consumed in the process.

Gametic (phase) equilibrium – the state of loci on different chromosomes when the allele at one locus in the gamete varies independently of that at the other loci.

Gel – matrix (often agarose or acrylamide) used in electrophoresis to separate molecules.

Gene – the basic unit of heredity; a sequence of DNA nucleotides on a chromosome.

Gene frequency –the relative occurrence of a particular allele in a population .

Gene mapping – determination of the relative positions of genes on a DNA molecule (chromosome or plasmid) and of the distance, in linkage units or physical units, between them.

Genetics – the study of the patterns of inheritance of specific traits.

Genetic drift – random fluctuation in allele frequencies due to small population sizes (sampling error).

Genome – the sum total of an organism's genetic material.

Genome projects – Research and technology development efforts aimed at mapping and sequencing some or all of the genome of an organism.

Genophiler™ – an automated, objective system for reviewing and presenting DNA profiling data.

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

Guanine – a purine base; one of the four molecules containing nitrogen present in the nucleic acids DNA and RNA; designated by letter G.

Hardy-Weinberg equilibrium (HWE) –populations of organisms that are in HWE has no statistical correlations between any pairs of alleles within individuals in the population.

Heredity – the transmission of characteristics from one generation to the next.

Heterozygous – a heterozygous organism has two different alleles at a particular locus.

Homozygous – a homozygous organism has two copies of the same allele at a particular locus.

Identifiler – PCR Amplification Kit (AmpFLSTR® Identifiler™) that provides human identification laboratories with the ability to generate information on 15 STR loci and Amelogenin.

In vitro – outside a living organism .

Kilobase (kb) – unit of length for DNA fragments equal to 1000 nucleotides.

Kinship coefficient – the probability that two randomly chosen genes, one from each of two individuals in a population, are identical (i.e. both descended from the same ancestral gene, or one from the other).

Linkage – the association of alleles at two or more loci due either to their residing on a single chromosome or their abundance in a particular ethnic

group that causes them to appear together at a higher than expected frequency.

Localize – determination of the original position (locus) of a gene or other marker on a chromosome.

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

Marker – a gene of known location on a chromosome and phenotype that is used as a point of reference in the mapping of other loci.

Matrix failure (pull up) – a result of the inability of the detection instrument to properly resolve the dye colors used to label PCR amplification products. Often due to off-scale peaks.

Megabase (Mb) – unit of length for DNA fragments equal to one million Nucleotides.

Mitochondrial DNA (mtDNA) – DNA found in the mitochondria inside cells (not associated with the nuclear chromosomes); transmission is only from mother to child.

Mitosis – the process of nuclear division in cells that produces daughter cells that are genetically identical to each other and to the parent.

Multiplexing – a sequencing approach that uses several pooled samples Simultaneously, greatly increasing sequencing speed .

Mutation – any inheritable change in DNA sequence.

Nucleic acid – a nucleotide polymer that DNA and RNA are major types.

Nucleotide – chemical units that are strung together in long chains to make DNA molecules.

Nucleus – the cellular organelle in eukaryotes that contains the genetic material.

Oncogene – a gene, one or more forms of which are associated with cancer. Many oncogenes are involved, directly or indirectly, in controlling the rate of cell growth.

Physical map – a map of the locations of identifiable landmarks on DNA. Distance is measured in base pairs.

PCR (polymerase chain reaction) – an amplification process that yields millions of copies of desired DNA through repeated cycling of a reaction involving the enzyme DNA polymerase.

Peak height imbalance – a significant difference (usually 30% or more) in the amount of signal obtained for two alleles from a single STR locus that might be suggestive of more than one contributor to a sample.

Polymorphic – a locus is polymorphic if a population contains two or more detectable alleles.

Polymorphism – difference in DNA sequence among individuals. Genetic variations occurring in more than 1% of a population would be considered useful polymorphisms for linkage analysis.

Population – a group of individuals residing in a given area at a given time.

Primer – short preexisting polynucleotide chain to which new deoxyribonucleotides can be added by DNA polymerase.

Probe – single-stranded DNA or RNA of a specific base sequence, labeled either radioactively or immunology that are used to detect the complementary base sequence by hybridization.

Proficiency tests – tests to evaluate the performance of technicians and Laboratories; in open tests, the technicians are aware that they are being tested, but in blind tests, they are not.

Profiler Plus – PCR Amplification Kit (The AmpFLSTR® Profiler Plus™) that provides human identification laboratories with the ability to generate information for nine polymorphic STR loci and the Amelogenin locus.

Protein – a large molecule composed of one or more chains of amino acids in a specific order; the order is determined by the base sequence of nuceotides in the gene coding for the protein. Proteins are required for the structure, function, and regulation of the body cells, tissues, organs, and each protein has unique functions.

Random match probability – the chance of a random match; as used in DNA profiling, it is the probability that the DNA of a randomly chosen person has a DNA profile that cannot be distinguished from that observed in an evidence sample.

Recombinant DNA technologies – procedures used to join together DNA Sequences in a cell-free system. Under appropriate conditions, a recombinant DNA molecule can enter a cell and replicate there, either autonomously or after it has become integrated into a cellular chromosome.

Resolution – degree of molecular detail on a physical map of DNA.

Restriction enzyme – a protein that recognizes specific, short nucleotide sequences and cuts DNA at those sites. Bacteria contain over 400 such enzymes that recognize and cut over 100 DNA sequences.

Restriction fragment length polymorphism (RFLP) – variation between

Individuals in DNA fragment sizes cut by specific restriction enzymes;

Polymorphic sequences that result in RFLPs that are used as markers on both physical maps and genetic linkage maps . RFLPs are usually caused by mutation at a cutting site.

RFU (relative fluorescent units) – units of measure for the light intensity detected by a fluorescence detector, correlated with the amount of DNA associated with a particular STR allele.

Serology – a discipline that uses immunology to study body fluids.

Sequencing – determination of the order of nucleotides (base sequences) in a DNA or RNA molecule or the order of amino acids in a protein.

Sex chromosomes (X and Y chromosomes) – chromosomes that are involved in sex determination . In humans, XX corresponds to female and XY to males. In STR testing, typed at the amelogenin locus.

STR (short tandem repeats) – in DNA testing, a subset of polymorphic VNTR loci where alleles differ primarily in the number of times that a string of four nucleotides are tandemly repeated.

Southern blotting – transfer by absorption of DNA fragments separated in electrophoretic gels to membrane filters for detection of specific base sequences by raiolabeled complementary probes.

Stutter – PCR amplification products that are one or more repeat units less (or more) in size than a sample's true allele and arise during PCR because of strand slippage. Typically 15% or less of the height of the true allele.

Tandem repeat sequences – multiple copies of the same base sequence on a Chromosome; used as a marker in physical mapping.

Thymine – a pyrimidine base; one of the four molecules containing nitrogen Present in the nucleic acids DNA and RNA; designated by letter T.

Taq polymerase – a DNA polymerase (an enzyme) used to amplify a specific DNA Template in the PCR technique.

VNTR (variable number of tandem repeats) – in DNA testing, a polymorphic Locus where alleles differ primarily in the number of times that a string of Nucleotides are tandemly repeated. Widely used throughout the 1990's but largely replaced by PCR-based STR testing today.

List of Abbreviations

CCD	Charge – Coupled Device.
C E	Capillary Electrophoreses .
CODS	Combined DNA Index System .
DNA	Deoxyribonucleic acid .
DTT	Dithiothreitol .
EDTA	Ethylene di amine tetra acetate .
ISS	Internal Size Standard .
LDIS	Local DNA Index System .
NDIS	National DNA Index System .
PCI	Phenol Chloroform Isoamylalcohol .
PK	Proteinase K.
PCR	Polymerase Chain Reaction .
RFLP	Restriction Fragment Length Polymorphism.
RNA	Ribonucleic acid .
RFU	Relative fluorescent unit.
SDIS	State DNA Index System .
SDS	Sodium dodecyl sulfate .
STR	Short tandem repeat .
TE	Tris ADTA.
VNTR	Variable number of tandem repeat .