



Principles of Genetic Fingerprinting in Forensic Medicine

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مبادئ البصمات الوراثية في الطب العدلي

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ABSTRACT

The examination of forensic DNA is the focus of this study. The analysis done in forensic labs' biology section is known as forensic biology. This essay aims to provide a brief overview of forensic DNA analysis and DNA categorization. The laboratory process known as DNA fingerprinting uses the nucleotide sequences of certain areas of human DNA that are unique to each individual to determine a person's potential identification. Paternity testing, other forensic applications, and forensic DNA fingerprinting investigations may all be used. The objective in these situations is to "match" two DNA fingerprints, such as a DNA sample from a known individual and one from an unknown individual. DNA evidence is simple to get since every human cell has genetic material. Every individual leaves a biological trail when they come into touch with living and non-living objects, making it possible to identify and relate it to the locations where Maha was born and grown. This information may then be employed in forensic science. With the ability to extract enough DNA from even the tiniest biological sample, police may now match suspects to evidence collected at crime scenes, defend the innocent, and catch the genuine offender.

Key words:

Forensic ,DNA marker , DNA Fingerprint, SNP, STR,REFLP.

الخلاصة

موضوع هذا البحث هو وتحليل الحمض النووي العدلي. البيولوجيا الجنائية هي التحليلات التي تجرى في أقسام علوم الحياة في مختبرات الطب العدلي. الغرض من هذا البحث هو تقديم مراجعة سريعة لتصنيف الحمض النووي وتحليل الحمض النووي العدلي. يتم استخدام التسلسلات النوكليوتيدية لمناطق معينة من الحمض النووي البشري الفريدة لكل شخص في الإجراء المخبري المعروف باسم بصمة الحمض النووي للتأكد من هوية الشخص المحتملة. يمكن ان تستخدم اختبارات الأبوة وتطبيقات الطب العدلي الأخرى وكذلك التحقيقات الجنائية من بصمات الحمض النووي. في هذه الحالات، يكون الهدف هو "مطابقة" بصمتين من الحمض النووي، مثل عينة DNA من شخص معروف وواحدة من شخص مجهول، مع بعضهما البعض. نظرًا لأنه يمكن العثور على المادة الوراثية في كل خلية بشرية، فمن السهل جمع أدلة الحمض النووي. وبالتالي قد يتم التعرف عليها وربطها بالمواقع التي رفعت منها حيث يترك كل شخص اثر بايولوجي عند ملامسته الأشياء الحية و غير الحية وهذا يمكن الاستفادة منه في علم الطب العدلي. يمكن الآن استخراج كمية الحمض النووي اللازمة للتحليل من أصغر عينة بيولوجية، مما يمكن السلطات من مطابقة المشتبه بهم بالأدلة التي تم العثور عليها في مسرح الجريمة وإبعاد الاتهام عن الشخص البريء وتحديد المجرم الحقيقي.

الكلمات المفتاحية: الطب العدلي ، معلمات الحمض النووي ، بصمة الحمض النووي. SNP, STR, RFLP ,



1. INTRODUCTION

The name "forensic" originates from the Latin word *forensis*, which meaning "of or before the forum." The term has its origins in Roman antiquity, when a criminal case was resolved in favor of the party that presented the best case and did so in a forum in front of the largest possible audience. The term "forensic science" refers to the use of scientific knowledge to resolve legal disputes, both criminal and civil, since "forensic" is an adjective that signifies "pertaining to, or utilized in courts." Although "forensics" and "forensic science" are often used synonymously, they have a number of ambiguous definitions.[1].

For all currently recognized forms of life, biopolymers and large molecules known as nucleic acids are required. They are made up of nucleotides, which are monomers with the following chemical components: a nitrogenous base, a phosphate group, and a 5-carbon sugar. The two main forms of nucleic acids are ribonucleic acid and deoxyribonucleic acid (DNA) (RNA). The polymer will be RNA if the sugar is ribose and DNA if the sugar is deoxyribose. [2].

The primary information-carrying molecules in organisms and the genetic material are naturally occurring chemicals known as nucleic acids. Large quantities of nucleic acids are present in all living things and are used to construct, encode, and then store the information that is present in each and every living cell of every kind of life that exists on Earth. They subsequently help to communicate and express that information to the cell's internal mechanisms and, ultimately, to each living thing's progeny both within and outside the cell nucleus. The encoded information is stored and transmitted in the nucleic acid sequence, which gives the "ladder-step" ordering of nucleotides within the molecules of RNA and DNA. They are very important for regulating protein synthesis.[3].

DNA is an essential component of forensic research since it may be used to both exonerate the innocent and condemn the guilty. By processing and analyzing the biological evidence brought to the crime scene, the genetic information in DNA enables the identification of the perpetrator. [1]. These biological elements might include blood, saliva, semen, buccal or vaginal swabs, or even touch DNA. Sometimes the culprit is unaware since the chemicals are scattered in tiny amounts on a surface. Processing DNA from body fluid samples and DNA statistical analysis may be used to identify a person. This is done by swabbing known profiles from the crime scene and utilizing DNA databases like CODIS. If a known profile is unavailable, CODIS, which has reference profiles of prior offenders, may be used to search for the elusive profile.[1], [4]. The aim of the current study is brief overview is given of past and present DNA typing and the establishment of forensic DNA databases .

2. Review of Literatures

2.1. Forensic DNA Sources

Biomaterials such as bones, blood and bloodstains, semen and seminal stains, tissues, organs, teeth, hair, and fingernail samples are often utilized for DNA extraction and typing. The quantity of DNA that can be extracted from various common biological sources will vary.[5].

The human body is composed of billions of nucleated cells, with the exception of red blood cells. The two copies of each person's genome that are found in each nucleated cell may be used to construct DNA profiles. The majority of samples show some degree of degradation, but when this degradation is severe, more cellular material is needed in order to create a DNA profile.[6].

The examination of biological evidence has evolved dramatically over the past 15 years because to the forensic application of DNA typing tools. Due to its exceptional sensitivity and



discriminating power, DNA analysis has acquired popularity in the fields of forensic science, forensic medicine, anthropology, and paternity testing.[7].

A variety of physical evidence is often brought into forensic science laboratories for analysis. Initially, the only kind of evidence that could be utilized for DNA analysis was biological materials, including nucleated cells. This limitation has been lifted thanks to the use of mitochondrial DNA sequencing in the forensic profession during the last five years. [8].

At crime scenes, skin or mucosal cells may be left on objects like knife handles, keys, socks, or toothbrushes. This is referred to as contact DNA evidence.[9] . Since the first instance of touch DNA evidence was provided in 1997, there have been a considerable increase in the number of instances using touch DNA samples in forensic investigations. [10].

These materials are difficult to extract complete DNA profiles from, and sometimes need several amplifications, which may slow down the procedure as a whole. Since it may be difficult to identify cells or DNA on the acquired evidence, it is often processed blindly after being broken up into multiple little pieces. If too many samples are obtained from a single piece of evidence, the DNA concentration may be lowered, and contamination is a common issue. Numerous studies on touch DNA samples, including examinations of cell-free DNA, have been published.[11],

the effects of a person's age, DNA transfer, and preprocessing methods. One preprocessing method is often used in all scenarios, and the use of sticky tape and the double swab technique was investigated for the preprocessing method. [12]. Studies using the vacuum cleaner technique [12] When dealing with gunshot leftovers, China employs a vacuum cleaner method as a preprocessing procedure to collect leftover cells on contact samples before DNA extraction.[13].

2.2. Forensic DNA Collection

DNA profiles were taken from touched things more than 10 years ago. The theory and applications relevant to the transfer of DNA traces via skin contact were thoroughly examined by Wickenheise. [14]. DNA may be spread by touch in many different situations. They range from killings where the car's steering wheel formed a priceless profile to sexual assaults when the savagely imprisoned victim lost a contact lens. Further research led to the discovery of the contact lens from the vacuum cleaner's contents, and its DNA profile matched the complaint.[15]. Additional useful sources of DNA were gloves left at the scene of a crime and the outside ends of the electrical cable used in a strangulation. A study on the significance of DNA transfer in manual strangulation was just completed. [16].

The deposition of the biological components has been tried to be standardized in earlier touch DNA investigations, but none of them has been effective in producing a definite positive control. In earlier studies, participants collected touch samples by placing their hands on glass plates, sterile tubes, or the hands of other volunteers for a set period of time, such as 3, 10, or 60 s. [17]–[19]. Other research methods required subjects to wear a piece of clothing or rub their hands over a substance for a predetermined period of time. [20]. However, owing to the considerable inter- and intra-individual variability and the possibility that even a single person might display either exceptional or horrible shedding behavior depending on the circumstances in place at the time of sampling [17], The DNA that was deposited is not reliable.

It is difficult to understand how DNA is transmitted from the skin. Wickenheiser [14] gives an example of how skin cells form. Some authors claim that skin epithelial cells may be visually distinguished from other epithelial-type cells present in the mouth and vagina by their lack of nuclei and keratinization. [21]. According to Wickenheiser [14], the sloughing process exposes



skin cells to significant numbers of DNA-bearing cells as they travel to the skin's surface. This might add DNA to skin epithelial cells, which would otherwise not be a good source. Therefore, it may not be as straightforward to assign the biological origin of the DNA on the skin to skin cells alone. The Wickenheiser [14].

2.3. Forensics DNA Fingerprinting

Analysis of human genetic variations has enhanced forensic genetics. Prior to the 1980s, the main techniques for classifying (i.e., figuring out the kind of sample) and individualizing (i.e., figuring out the uniqueness) biological evidence were histology, microscopy, immunology, biochemistry, and serology applications [1].

Without without realizing it, we leave cells behind wherever we go, and almost all of them carry DNA. Saliva, hair, blood, and skin flakes all contain DNA that may be used to identify us. In actuality, law enforcement agencies and prosecutors from all over the world often use these little pieces of lost DNA to link criminals to the crimes they commit. This remarkable technique is sometimes promoted on well-known television shows as a simple, accurate, and reliable method of identifying criminals and prosecuting them [23].

2.3.1. Type of DNA Fingerprinting

2.3.1.1. Short Tandem Repeat (STR)

Despite the fact that the majority of the human genome is the same in every individual, there are some variations. This mutation may occur even in sections of the genome where protein coding regions are not known to exist. According to studies, these non-coding regions contain repeating DNA units that vary in length from person to person. Short tandem repeats (STRs), a particular kind of repeat, have been shown to be relatively easy to quantify and compare across different individuals. In actuality, the Federal Bureau of Investigation (FBI) has found 13 core STR loci that are currently often used in the identification of individuals in the United States, while Interpol has revealed 10 common loci for the United Kingdom and Europe. Nine STR loci have also been discovered in Indian populations [1].

Based on the different repetition units, STRs may be classified into a number of different groups. According to the size of the main repeat unit, STRs are classified as mono-, di-, tri-, tetra-, penta-, and hexanucleotide repeats. There are fewer instances of each category overall as the repetition unit becomes bigger. Dinucleotide repeats are the most frequent STR type in the human genome. Contrarily, STRs are divided into two types: perfect repeats (also known as simple repeats), which consist of a single repeated unit, and imperfect repeats (also known as complex repeats), which consist of several composition repetitions. [22].

2.3.1.2. Variable Number Tandem Repeat VNTR

Tandem repeats are abundant in the human genome. Tandem repeats were first categorized as a subset of minisatellites in the 1980s. The core sequence of several of these repeats contains a lot of GC. Later, tandem repeats with higher AT concentrations in the core sequence were also described. The minisatellites are sometimes referred to as variable number tandem repeats (VNTRs), as seen in Figure (2.2). The repetition unit length of a VNTR may range from a few to hundreds of base pairs (bp). The number of tandem repeat units at different VNTR loci varies greatly, and the arrays of tandem repeats may be kilobases (kb, or 10³ bp) long, resulting in DNA fragments of different lengths. The number of tandem repeat units found at a certain locus defines a genotype [24].

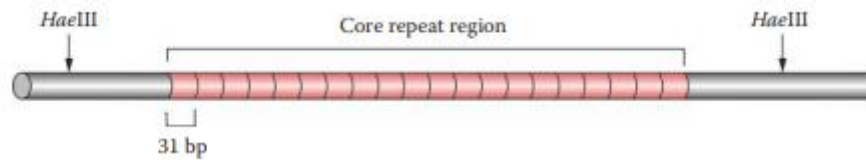


Figure (1) VNTR locus D2S44 (2q21.3–2q22). Each repeat unit consists of 31 bp. HaeIII represents the HaeIII restriction site [23].

2.3.1.3. Restriction Fragment Length Polymorphism RFLP

VNTR profiling uses RFLP, the first historical method for forensic DNA testing (Figure 2). Restriction endonucleases are used, which locate and cleave specific places along the DNA sequence. A recurring collection of restriction fragments with various lengths is created when a particular restriction endonuclease cleaves a DNA sample. In order to cut the genomic DNA at points that border the VNTR core repeat region, it is crucial to choose the proper restriction endonucleases. The resulting fragments are then separated according to their sizes by gel electrophoresis using a standard agarose gel. The DNA is then processed using the Southern transfer and hybridization procedure. The denatured DNA is subsequently moved from the gel to a supporting matrix, such a nylon or nitrocellulose membrane. The DNA that has been immobilized on the membrane will next be hybridized with a tagged probe. Autoradiography-based detection methods, for example, only detect DNA bands with sequences complementary to the probe [26].

The presence of fragments of different lengths may be utilized to detect variations in homologous DNA sequences after the relevant DNA samples have been digested with certain restriction endonucleases. RFLP is a certain clone/restriction enzyme pair-specific molecular marker. The bulk of RFLP markers are co-dominant and very locus-specific (both alleles in a heterozygous sample would be recognized). An RFLP probe is a labeled DNA sequence that, after being separated from one or more digested DNA sample fragments by gel electrophoresis, hybridizes with those fragments to produce a characteristic blotting pattern unique to a certain genotype at a particular locus. Short, single- or low-copy genomic DNA or cDNA clones are often used as RFLP probes. [24].

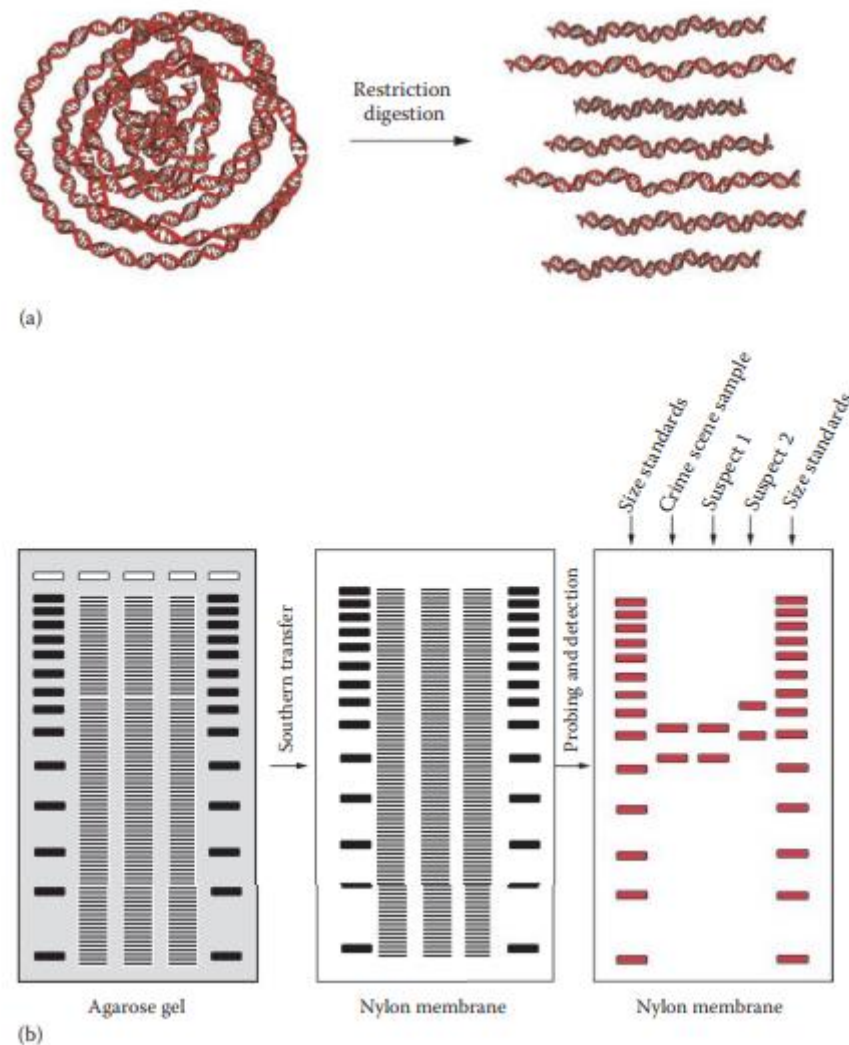


Figure (2) RFLP. (a) Restriction digestion generates restriction fragments with various lengths of genomic DNA. (b) Restriction fragments are separated by gel electrophoresis. DNA is transferred to a solid phase and probed. The signal is detected and the DNA fragment of interest can be observed. Band patterns of heterozygous loci of individuals are shown [23].

2.3.1.4. Amplified Fragment Length Polymorphism

The RFLP analysis of VNTR profiling fails when DNA from crime scene samples is degraded or inadequate. As a consequence, an improved VNTR approach was developed. Alleles at certain VNTR loci are just a little over 1 kb long. By using PCR, these loci may be amplified. This technique is known as amplified fragment length polymorphism (AFLP). One locus, D1S80, was used by forensic DNA laboratories for AFLP analysis [1].

Fragments between 14 and 42 repetition units (16 bp per repeat) were amplified using the AFLP method. The amplified DNA fragments were often separated by size using polyacrylamide gel electrophoresis, and their identification by a silver stain was common (Figure 2-4). Since



DIS80 loci were shown to be unique alleles, they may be directly compared to an allelic ladder (a collection of common alleles used as a reference) on the same gel. [27].

2.3.1.5. Y Chromosome Haplotyping

The Y chromosome, which is inherited from the father and passed on to all male offspring, is known as patrilineage. The Y chromosome is hence unique to males. On the chromosome, there are several genes that are necessary for male-specific activities such spermatogenesis and sex determination. The human Y chromosome genome includes roughly 59 million base pairs and contains 50–60 genes. The two subregions of the Y chromosome are the pseudoautosomal region and the male-specific Y region [28].

Applications of forensic DNA profiling that largely rely on Y chromosomal regions are covered in this chapter. For instance, Y-STRs may be used in forensic DNA testing and are useful in the investigation of sexual assault cases involving male suspects since they are male specific (for humans and other higher primates). In such cases, the evidence often contains high concentrations of female DNA and low concentrations of male DNA [29].

2.3.1.6. X Chromosome Haplotyping

X-chromosomal STR (X-STR) profiling is a useful technique for kinship testing in forensic investigations. Men normally have one X chromosome, hence they are hemizygous for the X-STR loci on this chromosome. The only PARs that can take part in homologous recombination between the X and Y chromosomes are those that are homologous. The paternal X chromosome's haplotypes are passed down to daughters [30].

As a result, detecting father-daughter kinship is easier when using X-STRs rather than autosomal STRs. In females, the X chromosome is present in two copies. Homologous recombination between two X chromosomes is conceivable during mother-child transmission. The accuracy of mother-child kinship analysis using X-STR is lower than that of father-daughter kinship analysis, notwithstanding the possibility. Sharing unusual X-STR haplotypes, however, may strengthen a kinship signal [31].

Many of the characteristics that the X chromosome contains are not shared by the Y chromosome, the X chromosome, or the autosomes of the mammalian genome. The different structural characteristics of evolution have led to the genetic differences that are now believed to be exclusive to gender. Males have a single copy of the X chromosome, which precludes recombination (except for the pseudoautosomal regions, PARs, which maintain homology by recombining during male meiosis). The non-recombining regions, the PAR 1 and PAR 2 sections, and the X and Y chromosomes have all followed separate evolutionary pathways and become highly differentiated as a result of having various functional functions, leaving just a few X-Y sequence similarities between them. [25]

2.3.1.7. Mitochondrial DNA Profiling

Forensic mitochondrial DNA (mtDNA) analysis enables the identification of victims, including those who are unaccounted for and those in mass mortality occurrences. These individuals may be recognized by comparing their mtDNA profiles to those of their maternal relatives since mtDNA is inherited from the mother. The nuclear genome is far less common in cells than the mtDNA genome [32].

Thus, mtDNA testing is often employed to assess evidence from materials like hair shafts that have minimal nuclear DNA. Additionally, nuclear DNA degradation in rotting tissues and buried bones may be evaluated using mtDNA analysis. MtDNA analysis is often carried out on materials obtained from skeletal or deteriorated remains. Clean the sample's surface to remove any contaminants or adhering material. Bones and teeth are pulverized up to facilitate the mtDNA extraction procedure [33].

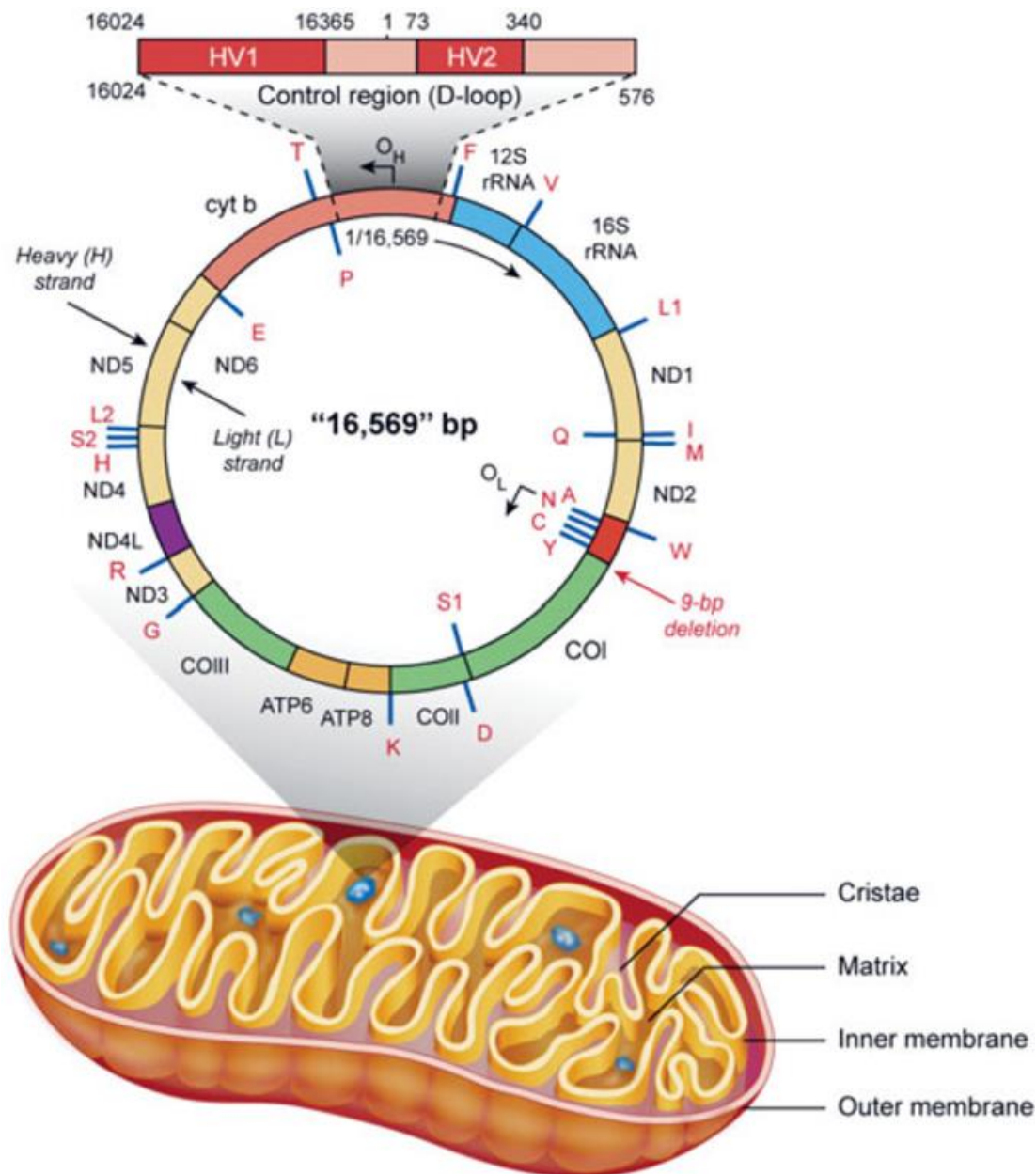


Figure 3. The 16569 kb human mitochondrial genome. The control area (D-loop), which contains the two hypervariable regions, HV1 and HVII, that are frequently employed in forensic DNA analysis, is enlarged at the top. [26].



2.3.1.8. Single Nucleotide Polymorphisms (SNP)

SNP is the abbreviation for single nucleotide polymorphism (SNP) which occurs at particular location as a single nucleotide variation in the genome. These variances are found to a significant extent in various populations and people. SNPs are present in every genome, including that of humans, some plants, and bacteria-like microbes. Due of their strong benefits in parentage testing, SNPs have attracted the attention of forensic researchers. SNPs are excellent for examining and identifying damaged samples with small amplicons since they have modest mutation rates. SNPs offer useful details on the geographic origin and unique identification of samples of unidentified people, plants, and microbes [27].

Conclusion

DNA evidence is straightforward to compile since genetic material can be found in all human cells, with the exception of red blood cells. By leaving behind tiny biological traces of ourselves, we may be identified and related to the places we've been. Authorities may now match suspects to evidence retrieved from crime scenes by extracting the necessary amount of DNA from even the tiniest biological sample. However, as forensics is a discipline that is mostly based on chance, even a verified "match" does not provide clear proof of guilt. The overall effectiveness of DNA databases designed to make it simpler to connect previous offenders to present crimes is also constrained by difficulties with individual genetic rights and issues with delayed sample entry. The RFLP is more accurate than the PCR because it uses a bigger sample size, fresh DNA samples, and there is no amplification contamination.

Advantages of STR The test costs less, uses fewer specimens, and takes less time to complete..

Conflict of interests;-

There are no conflicts of interest.

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