

Effective Utilization of Molecular and Other Tools in Forensic Identification

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Abstract - It is essential to identify the culprits in the criminal cases as normally it is hampered by the lack of identifiable evidences. The utility of advanced tools using genome of the individual provides accurate and effective identification process in forensics. There are plenty of molecular markers which are used for different purposes of forensics. In addition other tools such as advanced microscopic methods and facial recognition tools aids the confirmation of the suspects in forensic science. Here, we discuss the various molecular markers and other tools used in identification process in forensic science.

Key Words: Forensics, Molecular markers, SEM, Microscopy, Spectroscopy

1. INTRODUCTION

The word forensic is derived from the Latin word “forensis” meaning “of or before the forum. The term was introduced during Roman times in which the criminal investigation is done in front of a group of public in the forum. The investigation at times biased as it was only based on the arguments in front of the forum rather than the evidence [William. 2018]. In recent times, forensic science is also known as criminalistics, is the use of science and technology to solve the criminal cases by collecting, preserving and analysing the evidence from the crime scene. Many methods such as scanning electron microscopy advanced photocopying methods effectively used in forensics. Further, molecular methods provided much impetus to identify the suspect through minute biological materials from the suspects. [Horwell, 2014]. Wildlife forensic science is used to identify the wild species and solve the legal and illegal issues by the use of forensic methods. Humans hunt wild animals for direct consumption and their medicinal and ornamental value. It is also useful to stop the illegal poaching, smuggling and hunting of protected (endangered and threatened) animals. [Frankhan et, al., 2002]

2. MOLECULAR MARKERS USED FOR DNA FINGERPRINTING

DNA fingerprinting

DNA fingerprinting is a technique applied to establish a link between biological evidence and a suspect in a criminal case. DNA sample taken from a crime location is compared with a DNA sample from a suspect. If it matches, the involvement of suspect in the criminal case is established. Conversely, if the two DNA profiles do not match, then the evidence cannot have come from the suspect. Further, DNA fingerprinting is also used to establish paternity. DNA fingerprinting is a different set of features that are present inside the DNA, which give unique features identification of an individual. As fingerprints vary between the individuals, similarly DNA fingerprinting may also vary from individual to individual. [Access excellence. org: 2010]. DNA profiling has the following characteristics [Sullivan, 1994].

- Sensitive, data could be generated from tiny amount of even degraded biological samples.
- Capable of resolving mixtures of tissues from several individuals
- Possess greater power of discrimination between individuals—up to 1 million fold higher than other conventional techniques.

Simple Sequence Length Polymorphism

There is a short segment of repeated DNA sequence in the non-coding region of the genome. These repeated sequences may vary between individuals. This repetition gives rise to polymorphism which provide unique features. There are two types of repeated sequence / satellite DNA which are Microsatellites and Minisatellites.

Microsatellites/STR/SSR

Repeated sequences contain the repeated length of about 2bp to 6bp. The sequence which is present left and right of the repeated region is called flanking region or conserved region. The flanking regions are same in all human beings but the repeated region may vary from person to person. For example: - If a person having a CA repeats for 2 times in a

certain gene, this repetition will not be same in the another person at that location in the genome, but the flanking will be the same so that same primer could be used for testing any no.of individuals . This will give rise to DNA profiling. DNA fingerprinting is done by using PCR as it is faster and most accurate. STRs are repetitive elements dispersed across the genome. DNA extraction is performed first and PCR is done using the standardized STR primers. After amplification, automatic genotyping is done and analysed for genetic variation in terms of individual level. [Edwards et, al ., 1991]. If the repeated sequence contains the repeated length up 6bp to 25bp then it is called Minisatellite. This is also called Variable Number Tandem Repeats (VNTR).

Inter Simple sequence repeats (ISSR)

ISSR refers to the sequence which is present in-between the two SSR regions. The most important thing is the number of nucleotides present in between the two SSR regions can vary from person to person which could also be used in forensics for differentiation of individuals.

Single Nucleotide Polymorphism (SNP):

In SNP, single nucleotide change which may leads to polymorphism in an individual. These are the difference in the position in a genome where individuals can have different nucleotides in that particular position. This may give some unique features of an individual. SNP may potentially have 4 alleles. The disadvantage of SNP as it exhibit very less variability within the family [Butler et al ., 2007] SNP has less discrimination power than STR, needed to analyse simultaneously more than 100 polymorphic loci [Seo et al ., 2013] . Thus, it is used to identify the biogeographical ancestry of the sample [Pereira et al., 2017].

Sex specific Y chromosome as a marker (Y-STR)

Y chromosome is inherited from father to the all-male offsprings and codes for many genes which is responsible for the male specific functions includes sex determination and spermatogenesis. Y chromosome loci are also useful in forensics for identifying the sex of the suspect. Y chromosome is the 3rd smallest human chromosome which is slightly larger than chromosome no. 21. Y chromosome consist of PAR – Pseudo autosomal region, SRY – sex determining region and MSY – male specific region of Y. More than 400 Y –STR loci is identified in Y chromosome. The Y – STR loci located 60% at the long arm and 22 % at the short arm and few in the centromeric region. The identified Y – STR contains 60% dimeric repeats, 45% tetrameric, 39% pentameric and 1% hexameric. The most commonly used Y –

STR loci are DYS19, DYS385A, DYS385B, DYS3891, DYS3892, DYS390, DYS391, DYS392, DYS393 [Kayser. 2017]

RFLP analysis

DNA sample can be obtained from buccal smear, blood, saliva, skin, hair , body fluid , amniotic fluid and other tissues is used for analysis. Buccal smear is mostly used for analysis because it is non-invasive but it can be easily contaminated by bacteria, wherein blood sample yields good results. . Extraction of genomic DNA is done followed by the restriction enzyme is used to digest the DNA and it cleave the DNA in a specified restriction site into various sized fragments. These different fragments are then analysed using southern blotting.

Mitochondrial DNA

The most commonly used marker, mitochondrial DNA due to unique features useful for identifying the species. mtDNA is present in variety of samples such as old bones [Anderson et , al ., 1981] , teeth and hair [Ravikumar et al., 2018] and it is used to analyse when the DNA content in the sample is very low. mtDNA has its own rigid membrane with high content of protein which prevent the sample from degradation . There is a greater chance of mutation takes place in mtDNA than nuclear DNA due to the lack of proof reading activity. [Gray, 1989] [Saferstein, 2004] The most commonly used genes in mtDNA are cytochrome C oxidase subunit I, cytochrome b gene, 12s and 16s rRNA, D loop (In animals); rbcL and mat K (in plants).

E- DNA

In wildlife forensics, environmental DNA plays a major role in the identification of species. Environmental DNA samples can be collected from the water, soil, ice cores, trees. Using e DNA examination, we can able to find more than one species at a time. Environmental DNA obtained from water, soil etc., is used for amplifying using metabarcode primers and subsequently sequencing using high throughput sequencing tools. [Kowalski, 2015] The metabarcoding primers targeting the genes such as COI, 12s rRNA, 18s r RNA are commonly used for eDNA based approach in forensics.

1. OTHER MATERIALS USED FOR FORENSIC SCIENCE

Phenom Desktop SEM

Phenom Desktop Screening electron microscopy (SEM) is the most basic tool used in forensic sciences which acts as a high quality imaging tool and used to determine the composition of elements. It enables the scientist to solve the criminal

cases very easily and quickly. The residue which was deposited around the body of gunman after firing a bullet is called Gunshot residue, with this residue we can find whether the person was near to the crime scene or was holding the gun. This can be identified by using this Phenom desktop SEM with an energy dispersive X-ray spectroscopy detector (EDR). The scanning electron microscopy consist of column (for electron beam generation), specimen chamber (to hold the sample and the chamber can be tilted and rotated in all the sides), monitor and control panel. In the longitudinal section the flange leading to the high vacuum pump at the bottom. High vacuum is generated inside the microscope. This also consist of thin tungsten wire called thermionic cathode to create and emit electron into the vacuum and it is connected to the negative pole of the beam and anode is connected to the positive pole of the high voltage source. The strong electric field between cathode and anode generates the electron downwards (primary electrons). The beam of these primary electrons is not useful hence the electromagnetic lens is placed to focus it finely. By the use of this electromagnetic lens the primary electron knockout the electrons of the sample (secondary electron). This SEM also consists of Electromagnetic deflection, Raster scan generator and secondary electron detectors connected to the computer system. In this way we can magnify the image of the specimen.

Alternative light photography

In alternative light photography, an UV light and infrared light are used to see the evidence of the crime cases, which is not visible to our normal light. We can enhance the vision and bite marks by using UV light. Evidence such as physiological fluids like semen, urine and saliva can also be identified by their natural fluorescent properties with the use of UV light. Infrared photography will increase the intensity to magnify the blood, which makes us difficult to see due to clothing. The alternative light will brighten the substance to glow and darken the spot by absorbing the light and emit the wavelength of the substance when it reacts with the alternative light.

3D Scanner

3D Scanner is used in the crime site or room were drawn to analyse the crime in an effective way. 3D scanner will able to rotate 360 degree and take photographs of the entire room. 3D scanner not only takes photographs of the entire room but also measure the distance between the scanner and the wall, scanner and the objects which was present in the room. 3D scanner will give accurate readings within 10 to 30 minutes.

Ballistic photography (Moving under the force of gravity)

Ballistic photography is capturing the photos of the bullet which is moving under the force of gravity from the gun. Ballistic photography also involves pictures of splashing liquids or a balloon popping. In ballistic photography, the photographer need a trigger to line up the flash with the event as well as the cable release makes the camera to take the picture accurately.

Automated fingerprint identification System (AFIS)

Fingerprint varies from individual to individual. In forensic fingerprint is the most important key factor to solve the criminal cases. By AFIS, we can match the fingerprint of our sample with the known fingerprint in the database connected in the computer system.

Drug testing

Forensic officers need to identify the unknown substances in the crime scene. The substance may be in liquid or powder or pill form. In lab, they first undergo preliminary test such as colour testing, appearance to known that there is presence of substance or not. Then do some confirmatory tests like gas chromatography or mass spectrometry to know what kind of substance is present. However, Gas chromatography is used to separate single compound from mixture. Other tests are ultraviolet spectrometry (to note the reaction of the mixture by use of Infrared light and UV light) and Microcrystalline test (to know which type of drug is present in the mixture by the use of crystal pattern).

LA-ICP-MS (Laser Ablation Inductively Coupled Plasma Mass Spectroscopy)

This is the combination of a high temperature inductively coupled plasma (consist of free electron, positive ions and neutral atoms) and a mass spectrometry, the ICP source may converts the atom of the elements in the sample to ions and the separated ions are then detected by the mass spectrometry. A laser beam is focused on the sample, the material are then removed from the solid samples and gets ablated. The ablated particles are then passed into the ICP – MS instrument and at high temperature in the plasma the particles are digested and ionized then the particles move into the mass spectrometry for the chemical analysis.

2. CONCLUSION

Forensic science is a fast emerging field of study in the recent days. DNA fingerprinting technique helps to solve the

crime scenes and know about the evolution of a species and also involves paternity tests. In the most sensitive cases, suspect could be confirmed through Y chromosomal marker and by DNA Fingerprinting technique. By using different molecular markers we can identify the criminal involved in the crime scene by the traces left by them like hair, blood and several other evidences. Further, microscopic and spectroscopic methods are used to identify the suspects in the criminal investigations.

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