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Review: Recent Advances in Forensic DNA Analysis

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Abstract

Forensic DNA analysis is the use of DNA (deoxyribonucleic acid) in criminal justice testing. People can leave evidence behind when they commit a crime and normally they leave biological materials that contains DNA. If the evidence matches the latent print found at the scene of a crime, the match can provide evidence about the commitment of that person to the crime. Likewise, DNA recovered from stains of blood, semen, saliva, or from materials such as hair, bone and skin can be matched to DNA of a suspect. DNA can even be recovered from fingerprints.

Forensic DNA analysis or DNA profiling plays a major role in the criminal justice system. New techniques and technologies for DNA profiling continue to evolve every year. In this literature review recent advances in almost all the aspects of DNA analysis including sample collection, storage, pretreatment, DNA extraction, DNA quantitation, quality assurance of DNA testing, and DNA databases are discussed.

Keywords - Deoxyribonucleic acid, Forensic DNA analysis, Polymerase chain Reaction, DNA Profiling, Criminal justice testing

1. INTRODUCTION

Forensic DNA analysis or DNA profiling was first introduced in 1981 and since then it has been developed and has been established into a powerful tool for practicing criminal justice to crack the cases on crime scenes. Forensic science focuses on the use of genetic material in criminal justice system to answer questions related to legal concerns including civil and criminal cases. What makes DNA so useful and powerful is the uniqueness of DNA. While each person has 99.9% of human DNA sequences, the forensic scientists need only 0.1% of the DNA's unique sequences. This analysis plays a major role in the criminal justice system [9].

With subsequent adornment of DNA analysis methods in crime laboratories, even small amounts of blood, saliva, semen, skin cells or other biological materials can be used to develop investigative leads, to link a criminal or a victim to a crime scene or to disprove or confirm an account of the crime. Because of the correctness and fidelity of forensic DNA analysis, this evidence has also become an invaluable tool for an unlimited number of individuals who have been illegally convicted [9,10].

Typically, the following steps are performed during forensic DNA analysis:

- Sample preparation: DNA Is Collected and Prepared for Study, transported to DNA laboratory.
- DNA extraction: DNA extraction is the process by which scientists are able to separate the DNA and it is an important step, which provides information about the amount of DNA present in an unknown sample. This data can be used successfully to obtain better results preserving the sample for further analysis.
- DNA amplification: Artificial increase in the number of copies of a particular DNA fragment
- DNA quantification: To determine the average concentration of DNA present in a mixture
- DNA profile matching: This is the process of determining an individual's DNA characteristics

In this review, recent developments with particular emphasis on new techniques for manipulating and analyzing DNA are summarized [8].

Objectives of forensic DNA analysis: -

- To link an individual to a crime scene or to a criminal act: -

  Ex: Blood stains at a burglary crime scene

- To exclude suspects, persons of interest or consensual partners: -

  Ex: Sexual assault, obtaining husband's DNA sample for exclusionary purposes

- Other uses: -

- To identify human remains from mass disasters, unidentified bodies and missing persons

- Paternity testing

- To help further studies in recent advances in forensic DNA analysis

- To determine the chance that an individual is the actual source of the material that is being tested.

2. COLLECTION AND QUANTIFICATION OF DNA SAMPLES

Several factors can affect the molecular size of the DNA recovered from biological evidentiary specimens. One of the most common causes of DNA destruct is the action of the endo- and exonucleases that are common in nature. While the analyst can do nothing to prevent the destructive action of nucleases prior to specimen collection, there are several steps that can be taken to abrogate their activity once the specimen environment can be controlled. Specimens should be maintained cool and dry prior to the commencement of DNA recovery procedures.

However, Forensic scientists continue to appraise the success of sample collection methods, the sincerity of DNA samples, and storage of samples to certify accurate and reliable collection of DNA for further analysis. The cotton swab is a basic and essential tool for collecting DNA evidence for forensic analysis. However, a challenge to this analysis has been the quantity of the sample discovered from attest items for short tandem repeat (STR) analysis using the polymerase chain reaction. On the other hand, many factors can affect the recovery of a DNA sample, including the type of the sample, whether it is a sample of body fluids or epithelial cells, the type of evidence being checked such as skin, sexual assault kits, fingernails, and improvised explosive devices. [4].

One approach for determining if the biological etiology of an unknown sample could be semen, vaginal fluid, blood, saliva, feces or urine is the use of mass spectrometry. Mass spectrometry has become the method of choice for protein detection, identification and quantitation. The accuracy, sensitivity and flexibility of MS instruments have enabled new applications in biological research, biopharmaceutical characterization and diagnostic detection. Trypsin can be used to digest the sample to obtain the peptides that are present and then the peptides can be injected into a mass spectrometer. Biomarkers can be used to identify the type of the sample that is analyzed. This technique is not similar to other biochemical tests and is not specific to one type of sample.

Proper sample preparation for MS-based analysis is a critical step in the proteomics workflow because it can vary and can be time consuming. The quality and reproducibility of sample extraction and preparation significantly influence MS results. In addition, the mass spectrometer test can be added to the chelex or chelating step of DNA extraction, making it easily integrated into an already existing procedure. Another possibility for determining the biological origin of a samples is the use of Fourier transform infrared (FTIR) spectroscopy. Fourier transform infrared spectroscopy (FT-IR) has proven to be a valuable tool for the forensic scientist on the macroscopic level. That type of micro spectroscopy stretches the use of traditional FT-IR by allowing for quick, nondestructive analysis of samples approaching 10 microns. FTIR can be used as an indirect screening for DNA integrity when dealing with bone samples. Since the DNA in bones can be damaged due to excessive heat, FTIR spectroscopy is able to evaluate the bonds of collagen, which correlate with similar hydrogen and covalent bonds in DNA. Extracellular or cell free DNA has been found to exist in many biological media such as blood, saliva, semen and urine, which can easily be found in the supernatant of DNA samples during the DNA extraction process [9].

3. POLYMERASE CHAIN REACTION (PCR)

Developed in 1983, the process of PCR has made it possible to perform DNA sequencing and to identify the order of nucleotides in individual genes.

PCR stands for polymerase chain reaction, a molecular biology technique for amplifying segments of DNA, by generating multiple copies using DNA polymerase enzymes under controlled conditions. As little as a single copy of a DNA segment or a gene can be cloned into millions of copies, allowing detection using dyes and other visualization techniques. PCR techniques are applied in many areas of biotechnology including protein engineering,

cloning, forensics (DNA fingerprinting), paternity testing, the diagnosis of hereditary and/or infectious diseases, and for the analysis of environmental samples [3].

In forensics, repetitive DNA regions, which are located outside the coding regions of DNA, are used to further analyze DNA. These regions are different for each individual and can be used for identification of one person as well as a group of people, such as a group of family members. PCR is able to replicate specific nucleotide sequences from low levels of DNA or degraded DNA. The primers in PCR are specific to human DNA and results are not affected by bacterial DNA, if it is present. PCR uses a small amount of template DNA, two primers that flank the target sequence, nucleotides, and thermostable DNA polymerase to amplify a specific region of DNA, thus creating a large amount of DNA from a very small sample. PCR is extremely sensitive in that. Only trace amounts of the template DNA containing the sequence to be amplified are necessary in the reaction.

Some knowledge regarding surrounding sequences is needed, however to design the short, single-stranded, oligonucleotide DNA primers. Two primers are needed for PCR, one primer for each end of the sequence. The primers are complementary to the template DNA and confer the specificity of PCR for a target sequence. Which are then taken through a series of temperature changes. Products of amplification, or amplicons, are then separated using electrophoresis. The amplification process continues to be used in more advanced techniques, including being able to amplify just a sequence-specific region or a whole genome. The detection of DNA is often further evaluated with fluorescence, which uses fluorescent dyes that attach to PCR primers in the amplicons. Reagents used in the PCR process often consist of dimethyl sulfoxide, glycerol, formamide, single stranded DNA binding proteins and betaine.

Quantification of DNA can be greatly affected by PCR inhibitors, which can easily be obtained with a sample during DNA extraction. PCR inhibitors can then prevent the amplification process. Common inhibitors found in forensic samples include hematin, indigo, melanin, collagen, tannic acid, humic acid, and calcium phosphate [11].

4. DNA EXTRACTION METHODS

DNA extraction is an important step, which provides information about the amount of DNA present in an unknown sample. This data can be used successfully to obtain better quality results preserving the sample for further analysis. DNA extraction involves separating the nucleic acids in a cell away from proteins and other cellular materials. Different methodologies widely used by forensic DNA scientists include organic or solid-phase

extraction, post-extraction filtration and laser capture microdissection (LCM) etc. These methods are sometimes used to concentrate low amounts of recovered DNA [7].

Currently, one of the most frequently used methods of DNA extraction is organic extraction. Organic extraction (variations of phenol/chloroform) uses of a multistep liquid chemical process that is labor intensive but produces a high yield and very-clean double-stranded extracted DNA. In most of the other situations, a general method called organic extraction is usually employed. The organic extraction methods is more likely to maintain the DNA in large pieces, and clean the DNA more thoroughly than chelex extraction. The presence or absence of sperm, either alone with other types of cells, directs which variation of the organic extraction procedure might be performed. This method utilizes SDS and proteinase K to breakdown the cell membrane and proteolytic digestion, whereby the addition of proteinase K rapidly inactivates nucleases (e.g., DNases and RNases) that might otherwise degrade DNA during extraction. After lysing, the DNA is purified by mixing it with phenol-chloroform solution, centrifuged, and then the DNA is precipitated using ethanol and then resuspended in a low-salt buffer. The phenol-chloroform method is considered to be more effective when extracting high molecular weight DNA. Alternatives to the precipitation step have been also employed, switching to a filtration technique that could involve Centricon, Microcon, and Amicon filter devices that allow for increased DNA recovery and purification. A modified version of the organic method can be used when trying to selectively separate female and male DNA in sexual assault cases [9].

Another commonly used method of DNA extraction is the use of chelating resins that are based on ion-exchange chromatography. Ion-exchange chromatography is a process that allows the separation of ions and polar molecules based on their affinity to the ion exchanger. It can be used for almost any kind of charged molecule including large proteins, small nucleotides and amino acids. Most often the samples are added to a 5% solution of Chelex and then boiled for several minutes. The resins are able to bind to Ca2+ and Mg2+, deactivating unwanted nucleases and therefore preventing the cleavage of DNA. Non-polar nuclear DNA and RNA become denatured and stay in solution while polar components bind to the polar resin. The sample is then centrifuged with DNA present in the supernatant. The process of boiling the sample denatures the DNA and it is left with single stranded DNA, which results in having to use a PCR-based method to analyzed DNA. In addition, the purity of the DNA is not as good as compared to the traditional organic extraction or solid-phase

method. The manual Chelex method results in an increased number of PCR inhibitory components that are also extracted with the DNA. The PCR inhibitors cause problems in the subsequent quantification and short tandem repeat (STR) reactions used in DNA profiling [8].

Another method used is solid phase extraction (SPE). Solid phase extraction is a form of step-wise chromatography designed to extract, partition, and adsorb one or more components from a liquid phase (sample) onto a stationary phase (sorbent or resin). Over the last twenty years, SPE has become the most powerful technique available for the rapid and selective sample preparation prior to analytical chromatography. SPE extends the lifetime of chromatographic systems and improves qualitative and quantitative analysis. By switching sample matrices from the original matrix to a simpler matrix environment, subsequent analysis is often simplified, and the demand placed on an analytical system is considerably lessened. Solid phase extraction uses the difference of affinity between an analyte and interferents, present in a liquid matrix, for a solid phase. This affinity allows the separation of the target analyte from the interferents. An extraction process that continues to gain popularity in DNA extraction is the use of solid-phase extractions, which employs silica in the presence of chaotropic salts. The salts include thiocyanate, sodium iodide and guanidinium hydrochloride. Often the cells are lysed with proteinase K first, and then presented to chaotropic salt buffer to allow for the binding of DNA to silica. Once DNA is bound to silica, impurities such as proteins and other contaminants can be washed away. DNA can thereafter be eluted. Silica can be presented in a column fashion or as paramagnetic beads. The use of silica column does require a centrifugation step. Silica magnetic beads allows for a facile purification procedure that can result in high throughput extraction using robotic platforms. In addition, the magnetic beads can be used with many different sample types, including blood, saliva, and sperm with little cross contamination [13].

Another method often used in mixed samples is laser capture micro dissection (LCM). Laser capture micro dissection has become an indispensable tool for molecular analysis of extracted nucleic acids for molecular applications, including PCR of tumor DNA. LMD allows the isolation of single cells or small groups of cells out of routinely processed cytologic specimens. Today, several commercial systems are available to isolate cells like this. There an operator selects the cells of interest on a screen. The laser beam cuts the cells that are then placed in a microfuge tube, where extraction procedure for subsequent molecular analysis can be done directly. This method allows cells to be selected and collected by cell type, therefore

resulting in less cellular material needed. This method tends to work better with mixed samples where there is a minor contributor and a major contributor, such as when there is a large number of female epithelial cells in comparison to sperm cells in the sample. It also decreases the chances of mixed DNA profile results and the interference of PCR inhibitors. LCM methods are separated into ultraviolet (UV) cutting and infrared (IR) capture systems. The UV system can capture cells by photovolatilization. The IR capture system allows visualization of cells via microscope and then cells are isolated by laser energy to a thermolabile polymer [9].

In a mass disaster or forensic identification, where a dead body or its remains are highly decomposed or severely burnt, bone and tooth are often the only accessible source of DNA. Due to their unique composition and structure, DNA molecules in bone and teeth are largely protected from environmental challenges and/or biological attack. Bone is a connective tissue largely composed of collagen and inorganic mineral called hydroxyapatite. DNA has a strong affinity for hydroxyapaptite and its degradation is linked to the extent of crystallinity loss in hydroxyapatite as well as the loss of collagen. Bone density is another intrinsic factor in survival of bone material.

There is a significant difference in the bone density between men and women, and in different areas of the skeletal element morphology. A tooth on the other hand is composed of three major tissues, enamel, dentin and pulp. Enamel is the hardest tissue in human body being 96% mineral and contains no DNA. Dentins usually do not contain any nucleated cell bodies, other than some mitochondrial DNAs that accumulate from the odontoblastic process. Dental pulp is a highly vascularized connective tissue containing numerous cell types and rich in DNA. Since dentin and pulp is covered with enamel, this tissue provides a physical barrier protecting DNA within the tooth from external conditions like heat, sunlight, moisture and microbial attack. All bone and teeth DNA extractions involve two main steps, pulverization in liquid nitrogen and incubation with Ethylenediaminetetraacetic acid (EDTA). Most of the genomic DNA isolation methods use organic solvents involving phenol/chloroform, or silica-binding extraction from powdered bone or tooth materials. Mechanical grinding of whole tooth or bone requires a separate pre-amplification laboratory area and increase the risk of contamination from the dust particles.

Additional DNA extraction methods continue to be developed, addressing the issues of time, storage, and getting DNA from insoluble samples.

5. QUALITY ASSURANCE AND VALIDATION

A number of factors are required for the continuous evaluation and measurement of quality assurance and validation guidelines to help crime laboratories comply with federal standards and use new DNA analysis technologies. However, these factors include the pure power of DNA analysis to create extremely high protection potential, progress in the technology and techniques, a wide acceptation of DNA evidence in the courtroom, and the development of a number of commercial products for forensic DNA testing [9].

Forensic DNA laboratory can be separated into the prelaboratory, laboratory, and post laboratory. However, the pre-laboratory involves case assessment. The laboratory involves inspections, DNA quantification, DNA extraction, DNA amplification, electrophoresis and typing. Then the interpretation of results, databasing, and statement reporting are considered as post laboratory procedures. These steps are executed in different areas to avoid contamination and to make a smooth transition from one step to another [6].

6. ADVANCED AND EMERGING TECHNIQUES AND METHODOLOGIES OF DNA ANALYSIS

Since the advent of forensic DNA analysis in the 1980s, it has gone through several stages of development. Restriction fragment length polymorphism (RFLP) profiling is no longer used by the forensic community, as it requires relatively large amounts of DNA and degraded samples could not be analyzed with accuracy. The 2nd generation of DNA profiling included PCR techniques, which cannot be used in the profiling of longer strands of DNA. The current method of choice is short tandem repeat or STR analysis. It's called the 3rd generation of DNA analysis [10].

6.1. Short Tandem Repeat (STR) analysis

Short tandem repeat (STR) typing methods are used for human identity testing including forensic DNA analysis. At the same time, the human genome contains thousands upon thousands of STR markers, but only a small core set of loci have been selected for use in forensic DNA and human identity testing. Thousands of STR analysis are generated worldwide every year by universities, governments, and private laboratories performing different forms of identity testing for humans, including forensic casework, DNA databasing, missing persons victim identification, or parentage testing.

However, the short PCR product sizes of closely 200–400 bp generated with STR testing are generally compatible with degraded DNA that may be present due to environmental revile on biological material found at a crime scene [2,3].

6.2. Single Nucleotide Polymorphism (SNP) analysis

DNA can undergo different types of single nucleotide polymorphisms due to a change in one nucleotide in the sequence. Therefore, the DNA sequence will be altered. However, these Single nucleotide polymorphisms, constantly called SNPs, are the most common type of genetic differentiation among people. Every SNP introduces a difference in a DNA sequence by changing a nucleotide. Example, a SNP may replace the nucleotide thymine with the nucleotide cytosine in a certain draw of DNA. They happen once in every 200 nucleotides on average. However, it means there are normally 10 million SNPs in the human genome. They can perform as biological markers, helping scientists locate genes that are associated with diseases. When SNPs occur within a gene or in a regulatory region near a gene, they may play a more direct role in a disease by affecting the gene's function [2].

6.3. Mitochondrial DNA (mtDNA) analysis

Mitochondria are located inside the cells. However, every cell contains thousands of copies of mitochondria. Mitochondrial DNA are the DNA, which are located inside the mitochondria.

Mitochondrial DNA (mtDNA) has provided forensic scientists with an especial tool to determine the source of DNA recovered from degraded, damaged, or very small biological materials. mtDNA is a circular genome, which is located outside of a cell's nucleus [9].

The number of copies of mtDNA increases the likelihood of recovering measurable DNA from compromised DNA samples and for this reason, mtDNA plays a major role in missing persons investigations, mass disasters, and other forensic analysis involving samples with limited biological material.

Apart from the advances for low template DNA (LT DNA) that SNPs have made, alternative sources and methods continue to be looked into including mtDNA and singe cell analysis. MtDNA remains as a viable source because of its quantity. MtDNA is often used in LT DNA due to the higher proportional amount of mtDNA to nuclear DNA and its ability to be less prone to degeneration. The hypervariable (HV) regions of mtDNA are used for analysis due to their polymorphic characteristics and are valuable sources for analysis of degraded DNA including bone samples. The hypervariable regions are found to have the most variations. qPCR assay is highly specific and so it can be used in the mtDNA analysis. MtDNA analysis has also been paired with SNP-based screening methods providing a higher discrimination [5].

7. DNA DATABASES

Using DNA to identify people who are suspected to have committed a crime is one of the major advances in tackling crimes since fingerprinting. However, when DNA analysis is used clearly it can bring major benefits to society by helping to convict serious criminals including rapists and murderers. When information about genetic material extracted from a sample of tissues, and personal data are stored in a DNA database which can be used in forensic analysis it is defined as the National DNA Database (Schuster, 2005). However, there are fears that this information may be misused in ways that threaten our individual rights as well as those of our families. We must be confident that the government and the police use DNA in a way that complement our fundamental right to privacy and to protects civil liberties [14].

8. THE FUTURE OF FORENSIC DNA ANALYSIS

Development in DNA profiling was very slow until recently, but since 1985 more dominant techniques have increased in both number and quality explosively. However, the first useful system, the blood groups, was discovered and a half century later there were 17 blood group systems found, but not all were useful for DNA profiling, and after that a few enzymes and serum proteins were added. some 100 protein polymorphisms were known but most were not mainly useful for DNA analysis [1].

However, in 1986s there was a general breakthrough. VNTRs (variable number of tandem repeats) showed greater variability among people than previous systems and immediately began to be used for forensic studies. They are still used, but are rapidly being replaced by short tandem repeats [3].

Today, the general public is familiar with the fact that DNA techniques are being used. However, what may sound like “a simple test” is in fact the result of great efforts by a relatively new branch within the Forensic Sciences, one that has combined technological advancement, molecular genetics, statistics, and epidemiology into one: Forensic Biology.

Forensic DNA testing has played an important role in the criminal justice community through aiding conviction of the guilty and exoneration of the innocent. Remains from missing persons and victims of mass disasters have been re-associated and identified through linking reference samples to recovered remains. New technologies are regularly introduced and validated to expand the capabilities of laboratories working to recover DNA results with improved sensitivity and informativeness. Short tandem repeat (STR) typing continues to be

the primary workhorse in forensic DNA analysis although other genetic markers are used for specific applications [9].

9. CONCLUSIONS

In this review, a brief overview of the developments in the field of forensic analysis during the past years is given. New attains continued to be inquired for more effectiveness. However, even before DNA can be isolated, it is important to confirm the true identity of the forensic samples. Present techniques to determine the biological source of samples include, Fourier-transform infrared (FTIR) spectroscopy, mass spectrometry, and PCR. However, the effectiveness of different DNA extraction methods depends on the exact nature of the sample (such as urine, blood, sweat, semen, saliva, and bone etc.). However, there is a tradeoff between purity and quantity for DNA extraction methods. As an example, one tactic for high result DNA extraction was made possible by integral silica magnetic beads with an automated robotic stage. The method was successfully introduced to different samples such as blood, saliva, and sperm; however, it is not the best choice for samples with low concentrations of DNA. Fast Technologies for DNA Analysis were shown to reduce extraction time especially for blood or saliva. An enzyme-based microfluidic method can extract nucleic acid directly from whole blood, swabs, and blood found on cotton or denim.

Continuance is a negative factor in forensic DNA analysis as DNA start to degrade under normal environmental conditions. So preserving integrity of DNA for a long period of time and storage of DNA becomes a challenge. It has been established that DNA can be either dehydrated or kept in a specially-designed medium for long term storage. Apart from all available techniques to reduce DNA analysis time, which could also be cost effective, scientists continue to look at new techniques.

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