Forensic DNA Analysis: A Powerful Investigative Tool

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1.1 Introduction

The use of deoxyribonucleic acid, i.e. DNA, for the testing in criminal justice explains the term forensic DNA analysis in simple words. It was first introduced in 1981. The term forensis which is a Latin word has given birth to the forensic science where forensic means pertaining to; thus, the term forensic sciences means the use of various applications for the resolution of criminal disputes either criminal or civil. The DNA analysis has become an indispensable part of the modern forensic science; with the use of PCR techniques, it has become a major tool for the analysis of the biological material. The method of DNA analysis used in forensic science is also known by the popular term DNA profiling [1]. The main focus of this science is on the use of the genetic material in case of the criminal justice for solving the cases and answering the concerns related to the cases. It has become a very significant source for establishment and expansion of the databases of DNA collected from the suspected criminals. The technique of DNA analysis involves the use of genetic material in this process; the sample in the form of hair, skin and blood is collected from the suspects that are linked to a particular or various crime scenes [2]. Then the large numbers of isolated DNA sample are interrogated with the DNA databases in order to match the profiles of DNA at the scene of the crime. A DNA database comprises of the profiles of different DNA that are used for the analysis of genetic diseases, genetic fingerprinting or genealogy purposes.

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DNA carries genetic instructions in form of molecules of nucleotides in all living organisms. It is organized into chromosomes in a very structural manner. The nucleotide molecule of DNA comprises of phosphate backbone sugar moieties and nitrogen bases, namely, adenine (A), guanine (G), thymine (T) and cytosine (C). A double-helix structure of the DNA is formed by the attachment of the nucleotides in such a manner that they form two long strands [3]. Each individual DNA contains the specific information related to their heritage. The information gathered from the molecules of the DNA can help to find out certain diseases occurred due to mutations. Only 0.1% of the DNA is unique to each individual which is what the investigator looks for; the rest 99.9% of human DNA sequences are same in every person [4].

Every year, various new technologies are invented to bring out the best approach under the field of DNA profiling. The process of the DNA analysis is not one step; various steps are followed for the analysis of any sample collected. Some of the steps are preparation of sample and extraction of the DNA from the collected sample which is followed by amplifications of the isolated DNA [5]. After amplification, the DNA quantification in which different sizes of DNA present in form of fragments is separated, and at last, the DNA profile matching is done. Various techniques have been updated for the use of the genetic material in investigation. Some of them are restriction fragment length polymorphism (RFLP), polymerase chain reaction, variable number tandem repeat, Y-chromosome analysis, short tandem repeat (STR) analysis, low copy number analysis, single-cell DNA fingerprinting, touch DNA, mitochondrial DNA analysis, etc. [6]. From the past three decades, the development of all these techniques divided the process of analysis under different time periods and frames such as that during first decade only, the method of exploration was used for the analysis in which the utilization of the restriction fragment length polymorphism was done but at that time the analysis through PCR was not specific. Moving further by the second decade, the method of STR was on top for the DNA profiling in criminal cases as these genetic markers provide specific results from the distinct STR loci. Moreover, they have high degree of sensitivity through the PCR amplification. Then the rapid growth of the DNA databases lead to the use of the new Y-STR and use of STR kits on demand in the process of DNA analysis [7]. There are four different types of DNA databases, namely, forensic, genealogical, medical and national; each type of DNA databases has a main role of storing data, but under categories, they are divided. A forensic DNA database comprises of the data that is collected from the crime scene. The collected sample from the sites are sent to the forensic labs where the DNA profiles are generated. These profiles are stored in forensic DNA databases in order to use them for future investigations. They are widely used for the criminal investigations. They are also known as national databases as they are governed by the government. The first national database was developed in the United Kingdom in 1995 [8].

Genealogical DNA databases store the genome sequences which are first submitted by genealogists or geneticist. They are not used for cases that are not registered under police station; they are used more specifically for storage of the genealogical DNA test results. GenBank is one of the most widely used genealogical databases for

the storage of the genome sequences. In GenBank, the data is stored under different categories as per their species specificity. The information related to the genetic variations is stored in medical DNA databases. Such databases comprise of all the medical information about an individual related to their health, disease, variations in genome or other genetic variations. This information stored in medical DNA databases is very helpful in drawing a correlation between various diseases and the environmental factors or a correlation between diseases and the lifestyle of an individual. By this information, the generation of new drugs can be achieved [9]. Medical DNA database is used in forensics in cases where the relation between diseases and the victim is need to be found in order to clarify the case in a better manner. Each database plays a great role in the forensic science as without history of profiles of DNA the comparison of new profiles generated from the suspect would be difficult to analyse to generate end results. Moreover, with the storage of different DNA profiles, we can figure out even if the same culprit or one single person is responsible for two or more crime, and also it will also save time for solving cases. Various techniques have been implemented so that DNA profiling can be used in forensic science effectively. Even the degraded and decomposed samples can be collected, and isolation of the DNA can be done from them. With advancements in science and technology, now the use of nanotechnology has been introduced in combination to forensic science so that new portable devices can be manufactured and the cases can be solved in less time at the crime scene [10]. Nanoparticle such as gold nanoparticle has a quality to attract the charge present on the DNA towards itself so generation of biosensors in combination with the nanoparticle will be a great idea to be utilized in forensic studies to generate portable products. Currently, various techniques of the DNA fingerprinting that are RFLP, VNTR, STR, SNP, low copy number and Y-chromosome analysis are used to solve cases.

1.2 Developments in Forensic DNA Analysis

In early 1990s, the only method used involves the utilization of the blood groups and serum proteins isolated from the crime scenes; in addition to this, the use of various electrophoretic techniques was the main focus for the study in forensic science for solving criminal and rape cases. Various techniques were used under the blood grouping such as ABO typing, MNS system and Rh factor of a person, but due to the high probability of similar blood groups in a population globally, it led to decrease in efficacy of these conventional methods [11]. In addition to this, the markers used in this method of analysis were isoenzyme markers or proteins, but in these techniques, the problem was that the DNA isolation was not successful from that of the highly degraded or decomposed samples collected from the site of the crime. The advancements in DNA profiling increased extensively after 1985; the modern techniques of the forensic science evoke from the first application taken from the work of Alec Jeffrey. In 1985, while working on the myoglobin gene, Sir Alec Jeffrey from the University of Leicester, UK, introduced the new modern technique of the DNA fingerprinting that can be used for solving various cases in a short time

period. He named the repeated sequence of the nucleotides as variable number tandem repeat after his observation that he made in regard to them. He observed the repeated sequences in a specific combination in a nucleotide. Further, he found out that these repeated sequences differ from person to person, thus being helpful to determine clearly about the various DNA sequences and their human link. He termed this method as multilocus testing. He used restriction fragment length polymorphism to solve a rape case. RFLP is used for the analysis of the distinct fragments of the DNA of different sizes. In this method, a restriction endonuclease is used to cut the DNA fragments, and further, with the help of Southern blotting, the location of the repeat sequences is established [12]. Later in 1990, another effective technique for DNA analysis was developed, namely, STR, i.e. short tandem repeat. It is also known as microsatellites and simple sequence repeats (SSRs). They comprise of 2–6 base pairs, short sequences. They are very helpful in representing the discrete alleles that are not identical to each other [13]. But these approaches fail to deal with the problem of the small samples of the DNA as small samples give poor quality of the fingerprints, thus leading to negative results. This issue was resolved by the low copy number analysis which was developed by the UK forensic science service in 1999. Earlier to this, in 1997 at Australian Genome Research Facility, another successful development of the single-cell DNA fingerprinting method was invented by Dr. Lan Findlay. This technique was less time-consuming as compared to the others [14]. Currently, analysis through restriction fragment length polymorphism (RFLP), short tandem repeat (STR), variable number tandem repeat (VNTR), dot blots of allelic sequence information and mitochondrial sequence determination are widely used, and results are acceptable. A broad range of specialists work under the one field that is forensic science; some of them are criminalistics. They use logical and critical thinking for the investigation of cases, digital evidence analysis, expertise for fingerprint, dentistry, odontology, nursing, pathology, and toxicology under which various substances used by criminals to attack on victim are studied and questioned documents are maintained by the investigators to keep a record of every individual (Fig. 1.1).

Currently, introduction of nanotechnology to the forensic science is under process, and various methodologies are adopted in order to save time. Moreover, a single integrated platform for the extraction, amplification and sequencing of the DNA has already been developed with the help of microfabrication of capillary electrophoresis, but validation of such techniques is still under the process in order to utilize them freely in forensic sciences for investigation purposes [15], such as the development of Sci-Fi, a handheld device that can be taken to the crime scene. It is defined as a lab on the chip; the chip would be enough to test the samples at the crime scene in order to generate their sequences of the DNA. This method will provide great advantage as the number of samples can be tested at a single time and place; moreover, it is less time-consuming. Morphological analysis of the skull using three-dimensional computer automated techniques is under study in field of the forensic biology. In addition to this, determination of the colour of skin, hair and eyes with the help of the various techniques of gene sequencing is under the next-generation technologies of the DNA fingerprinting. Use of virtual autopsy, that is, virtopsy, is in

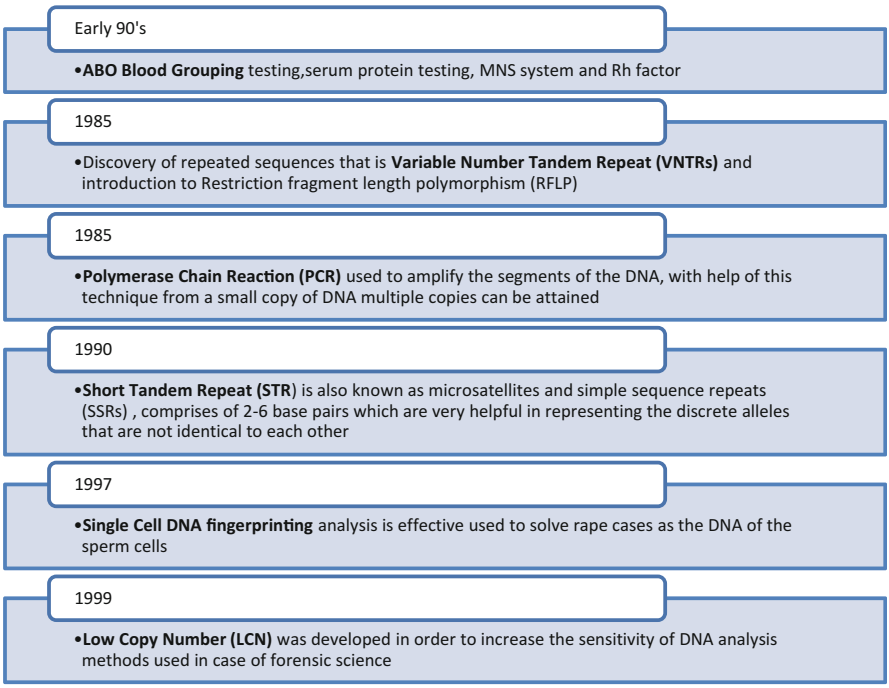

Fig. 1.1 Developments in various techniques of DNA analysis used in forensic science

the near future in coordination to the forensic biology. In this method, the collection of the images will be done [16].

1.3 Steps in Forensic DNA Analysis

The entire process of the DNA analysis is divided into four major parts, namely, serology, which further comprises of collection of sample followed by storage and its characterization. After serology is biology in which the isolation of the DNA is carried out; this involves extraction, quantification and amplification of fragments of the DNA and STR markers. The next step requires the technological aspects in which separation or detection of the DNA is done. After this, interpretation of the data is carried out to determine the characteristic of the isolated DNA from the sample [17]. At last, the role of genetics is there in which the statistical interpretation is done. This helps in the final analysis of the sequence of the DNA isolated from various samples. The genome sequence is compared with that of database DNA or with the suspect's DNA extracted so as to find out the actual culprit behind the crime. The detail process is explained as follows:

1. **Serology**: This is the initial step of any DNA analysis. In this step, firstly, the investigators report the crime site registered by police administration. Under this process, further collection of items, photography of crime scene, storage of samples like semen, hair, skin patches, blood or saliva, is done [18]. Further, after sample collection, the storage of them is mandatory in order to carry out any future investigations if required.
2. **Biology**: The next is based on the biotechnological aspects in which the collected samples are taken transported to forensic labs where the extraction of the DNA is done from both the victim and the suspected samples collected. After extraction, the quantification of the DNA is done in which the average concentration as well as the purity of the DNA are estimated. For the process of the quantification, spectrophotometer is used in which a fluorescent dye is used such as ethidium bromide or SYBR green dye is added to the samples; then, the samples are run on an electrophoretic chamber. The separated bands are visualized on transilluminator or on the gel documentation system [19]. The desired fragments of the DNA are amplified with the help of polymerase chain reaction in which from a small copy of DNA, multiple copies can be attained by using various enzymes such as DNA polymerases. DNA polymerases can be isolated from various organisms such as from bacteria. *Thermus acquaticus* generates Taq enzyme, similarly Pfu enzyme, from *Pyrococcus furiosus* and vent from *Thermococcus litoralis*. This process of amplification of DNA is done under controlled conditions. In the case of forensic science, PCR plays an important role for the identification of the repetitive DNA region [20]. After amplification, with the help of the STR markers, the regions are located, and the final analysis between the two sequences is done.
3. **Technology**: In this process, the separation and the detection of the isolated DNA sequence are done. The human and non-human DNA is separated so that the comparison of the other sequences from database can also be done to find out the person responsible for conducting a crime [21]. With the help of the bioinformatics tools and new technologies, the genome sequences are compared. GenBank is one of the most widely used genealogical databases for the storage of the genome sequences. In GenBank, the data is stored under different categories as per their species specificity. Thus, it helps to distinguish between various sequences of the DNA.
4. **Genetics**: In this step, the statistical interpretation of the collected data is done, and a final report is generated. The report contains all the information starting from the registered date of the case to the final report analysis. The information in a report consists of the photographs from the crime scene taken as evidence, list of samples collected as evidence, reason why the a specific person is taken as suspect and analysis of the DNA genome sequence that shows the matching of the two sequences giving clear information about the accused [22] (Fig. 1.2).

All the above-mentioned steps are carried out in forensic DNA analysis in order to solve the cases related to the criminal, paternity, and mass disaster and rape cases. The three possible outcomes are expected from the results obtained that include

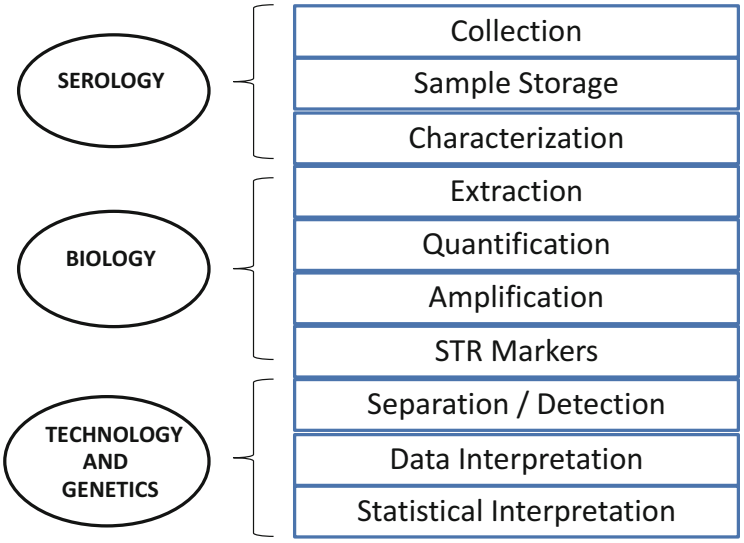

Fig. 1.2 Overview of steps involved in DNA testing

exclusion, inclusion and inconclusive results. Various techniques are discovered for the proper determination of the samples; some of them are low copy number, restriction fragment length polymorphism (RFLP), short tandem repeat (STR), variable number tandem repeat (VNTR) and mitochondrial DNA. Some of them are discussed as follows.

1.4 Various Techniques of DNA Profiling

- Restriction fragment length polymorphism (RFLP)
- Polymerase chain reaction (PCR)
- Short tandem repeats (STR)
- Low copy number (LCN) analysis
- Variable number tandem repeat (VNTR)
- Y-chromosome analysis
- Single-cell DNA fingerprinting
- Mitochondrial DNA (mtDNA) analysis
- Single nucleotide polymorphism

1.4.1 Restriction Fragment Length Polymorphism (RFLP)

This technique is used for the analysis of the different sizes of the DNA that are present in the form of small fragments. These fragments are formed by a digestion enzyme which is restriction endonuclease. It cuts the sequence of the DNA at a

specific site. The fragments obtained after the enzymatic reaction are separated using agarose gel electrophoresis. Furthermore, the fragments are separated by the technique of Southern blotting. Then with the help of multiple probes, the repeated DNA sequences are labelled with radioactive isotopes such as P^{32}. In place of radioactive isotope, a chemiluminescent dye can also be used. The probes can be either multilocus or single-locus probes as per the analysis requirements [23]. The difference in the DNA sequences present in a homologous manner can be detected with the help of RFLP. Most of the RFLP markers are highly specific in nature and work well under codominance conditions. Moreover, the method of development of the RFLP probes is quite simple. This technique was first used by Alec Jeffrey in 1985, when he was approached by the police; they convinced him to help them in the investigation of a rape-homicide case. This technique is effectively used in studying the evolutionary relationships, wildlife migration and breeding pattern in case of animals and for the detection as well as diagnosis of certain diseases [24]. As for the detection of various disease, the researcher can collect the DNA of family members so as to draw a comparison and find out the location of the affected gene and similar patterns of inheritance if occurred.

1.4.1.1 Advantages of RFLP

- A wide range of detection and diagnosis of diseases can be done.
- Specific for locus, thus helping in detecting the gene responsible for a particular disease.
- The evolutionary relationships can be studied.
- Highly reproducible in nature.
- Codominant markers making them species-specific.
- Utilize in genome mapping.
- No prior information on DNA sequence is required.

1.4.1.2 Despite of the Advantages of this Technique, Some of the Demerits Are

- Slow and tedious.
- Requirement of large amount of DNA sample.
- The quality of DNA should be good.
- Expensive technique.
- Radiolabelled probes are required.
- Probes are not available for every species.

1.4.2 Polymerase Chain Reaction (PCR)

This technique was developed in 1985 by Kary Mullis. It is a molecular biology approach that is used to amplify the segments of the DNA. With the help of this technique from a small copy of DNA, multiple copies can be attained by using various enzymes such as DNA polymerases. DNA polymerases can be isolated from various organisms such as from bacteria. *Thermus acquaticus* generates Taq

enzyme, similarly Pfu enzyme, from *Pyrococcus furiosus* and vent from *Thermococcus litoralis*. This process of amplification of DNA is done under controlled conditions [25]. This technique has a wide range of applications in the field of biotechnology; it can be used in genetic engineering, protein cloning methods, forensic DNA fingerprinting and paternity testing. In addition to this, it is effectively used in the analysis of various environmental samples. In the case of forensic science, PCR plays an important role for the identification of the repetitive DNA region [26]. These repetitive DNA are present outside the coding regions of the DNA. They are different from individual to individual. The process of the amplification requires two oligonucleotide primers; these primers are designed in such a manner that they are able to hybridize the opposite strands of the target sequence.

1.4.2.1 Polymerase Chain Reaction Method Is Carried Out as Follows

1. **Initialization:** This is the very first step in which only DNA polymerases are required, as they are needed to get activation by heat. For this purpose, the heating chamber is raised to 94–96 °C. Sometimes, in the case of thermostable polymerases, the temperature is increased to 98 °C for 2–10 min [27].
2. **Denaturation:** In this step, the hydrogen bonds break which are present between the two complementary bases of the DNA molecules, thus leading to melting or denaturation of the DNA. Temperature 90–98 °C is maintained for 10–20 s [27, 28].
3. **Annealing:** In this step, temperature is lowered so as the primers can anneal to the single stranded templates of the DNA. For 20–40 s, the temperature is maintained at 50–65 °C. The temperature should be specifically maintained as it should not be too low or too high; a moderation is required so that hybridization of the primer can occur on the specific target of complementary strand. In this step, the DNA formation begins when polymerase binds to the primer hybrid template [28].
4. **Extension and elongation:** In this step, the addition of the free deoxynucleotide triphosphates (dNTPs) from the reaction mixture; these are complementary to the template in the 5′-to-3′ direction. An optimum temperature is used for the thermostable Taq DNA polymerase enzyme which is 72 °C for 3–15 min for the last cycle of the PCR. After this step, the temperature of the chamber is decreased to 4–15 °C. This stage is termed as final hold; the main purpose of lowering the temperature is to cool down the reaction chamber [27].

This technique is highly preferred for the forensic analysis when the sample is minute or damaged as the amplification of DNA can be achieved by using this method and further analysis can easily be carried out. For instance, a rape case was registered in police under Indian panel code, the victim was brutally gang-raped, the sample was collected to confirm the criminal, and a real-time PCR was performed for the quantification of the DNA samples at State Forensic Science Laborartory. It can also be used for the detection and diagnosis of various diseases in such a manner that we can find out whether the disease has occurred due to mutations or some sort of inheritance [28]. Preimplantation diagnosis is widely used in case of in vitro fertilization approach as this helps in the prevention of defective births of neonatal.

Table 1.1 Various advantages and disadvantages of PCR in DNA analysis

Advantages	Disadvantages
Replication of specific nucleotide sequences from low levels of DNA or degraded DNA	Requirement of special markers that are specific for locus
Creation of large amount of DNA from a very small sample	Lower specificity towards culture or staining
Detection of diseases	Costly protocol
Require small sample for analysis	Chances of contamination
Less time-consuming	Possibility of amplification of unknown flora

Two widely used techniques for the process of DNA profiling under PCR are allele specific oligonucleotide and amplified fragment length polymorphism.

- **Allele specific oligonucleotide (ASO)**: It is short sequence oligonucleotide of 15–21 bases of nucleotides which is synthetic in nature and complementary to the sequence of the variable target DNA. In case of molecular techniques such as Southern blotting or dot blot which are effectively used in forensic science investigations, it acts as a source of probe. It is used for the diagnosis or detection of diseases such as sickle cell anaemia, which is caused by an altered mutation in the codon region [29]. In ASO, a complementary region is prepared to the test region in order to diagnose the disease.
- **Amplified fragment length polymorphism (AFLP)**: It is a technique used to detect various polymorphism among the different genomic regions. It was demonstrated in 1993 by Vos and Zabeau. It is used for the identification of various variations in genetics in same or distinct strains. In a single time frame, AFLP has a great capacity to amplify 50–100 fragments in one go. In addition to this, it is highly preferable technique for the analysis in criminal, paternity testing and generating linkage maps for the process of further quantitative trait analysis [30]. The process of amplified fragment length polymorphism involves the cellular DNA digestion with the help of some restriction enzymes followed by ligation of site specific adapters to those restriction fragments. The next step is the amplification of the fragments by the use of primers which are corresponding to adapters and restriction sites. At last, gel is run over an electrophoretic chamber to obtain bands which can further be visualized [31]. This method of DNA analysis is widely used in the study of various taxa; the main advantage of this is if the genomic makeup is not known, still one can do analysis and study of taxa by using this approach. Some of the demerits include the development of the locus – specific markers for the individual fragments are difficult (Table 1.1).

1.4.3 Short Tandem Repeats (STR)

They are also known as microsatellite and simple sequence repeats (SSRs). Just as variable number tandem repeats, the STR are short sequences of 2–6 base pairs long.

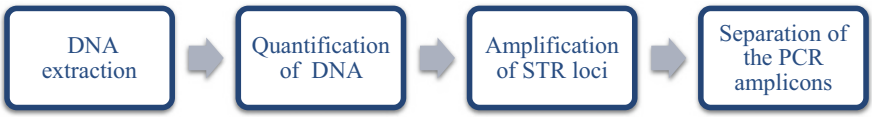

Fig. 1.3 Steps involving in DNA profiling through the process of STR are explained

In 1990, this technique was successfully used in forensic DNA analysis for the investigation purpose as they represent those alleles which are distinguishable from each other. In case of evidence, loci is stable, and even small amount of sample can be used as a short length of fragments is required [32]. This technique of DNA analysis in forensic science requires the use of polymerase chain reaction (PCR) for the process of amplification of short tandem fragments. STR is widely used in genetics for the construction of the linkage maps through linkage maps; diagnosis of genetic disorders can be done. STRs are divided on the basis of the length of the repeats as mono-, di-, tri-, tetra-, penta- and hexanucleotides. Due to the polymorphic nature and loci specific of the STRs, they are considered by the manufacturers to be in kit (330). They vary in size from person to person; such repetitive sequence does not affect the genetic health of the individuals. Mostly, they are found in non-coding regions, but in case of coding regions, they are even less than 10%. Special codes are used for the representation of the STRs, for example, D13S317; in this, D means DNA, 13 is the chromosome number on which the STR is located, and S stands for STR while the unique identifier is 317 (Fig. 1.3).

The very first step is the isolation of DNA by a process called DNA extraction, which is followed by the quantification of the DNA in the sample and at last the separation of the PCR amplicons [33]. The separation of amplicons is done on a genetic analyser by the utilization of bioinformatics tools that help to analyse the resulting data and compare the data from one specimen to databases which has the housing previously generated STR sets, thus helping in the final determination of the criminal among the suspects under study from the crime scene.

For investigation purposes, the samples in the form of bloodstains, semen or some biological traces from the victim's body are collected from the crime scene. This collected sample is investigated by forensic scientist in order to use such evidences for tracing the criminal by comparing the DNA profiling reports with databases of the DNA; after this analysis, the criminal can be found easily if the profile matches [34]. For instance, in Fig. 1.1, the sample from the crime scene was collected and then compared with the two suspects. From the analysis, it has been observed that the suspect 2 DNA profile shows repeat sequences of the STR loci which were identical to the evidence (Fig. 1.4).

The above diagram clearly explains that the repeated sequences were observed in case of sample 2; they are matching with evidence collected. Thus, this is how the process of short tandem repeat helps to solve the forensic science cases in order to trace the main criminal of the scene [36].

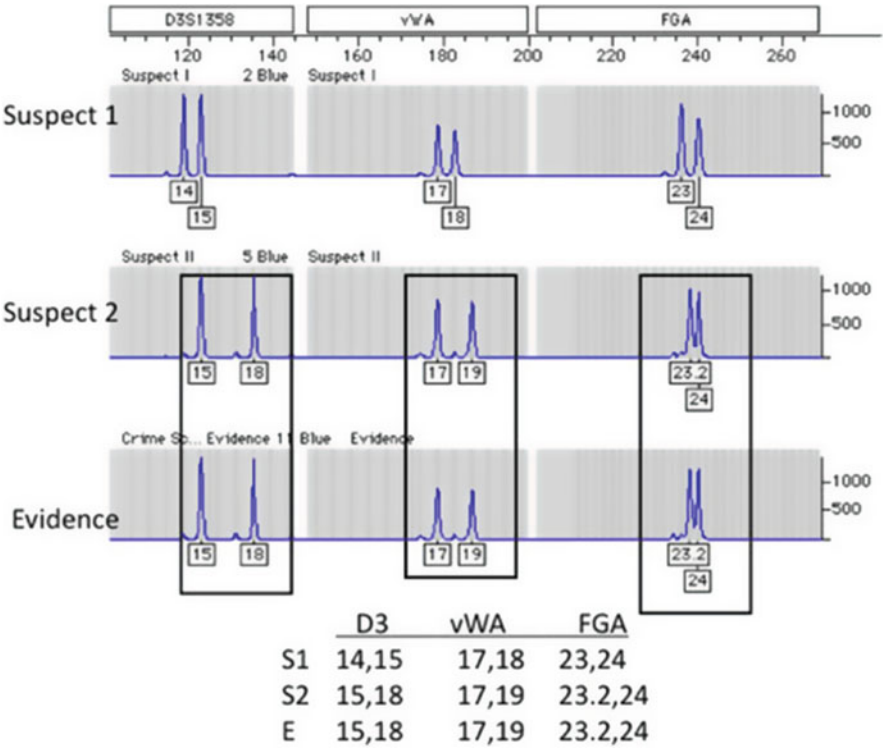

Fig. 1.4 The repeat sequence of Samples collected and the evidence [35]

On the Basis of the Pattern of Repeats, the STR is Divided into Following Categories:

1. **Simple repeats**—they contain similar length and sequence of the units.
2. **Compound repeats**—they are formed by combining two or more simple repeats.
3. **Complex repeats**—multiple repeat blocks of variable length of the units with intervening sequences are present.
4. **Complex hyper variable repeats**—due to allelic nomenclature issues, they are not widely used in forensic studies. The alleles differ in size and sequence, thus making it difficult for genotype reproducibly [37].

Various Applications of Short Tandem Repeats are as Follows:

1. They allow multiplexing due to narrow size of alleles.
2. From degraded DNA samples, the information can also be recovered by using STR method as they only require short repetitive sequence to analyse information.
3. The chances of mutation are low in this method.

4. Use of separate chromosomes in STR markers makes the technique more simple and unique, thus preventing any problem related to linkage between the markers.
5. They are highly preferred in DNA profiling as they can easily amplified by using PCR techniques without any complications.

1.4.4 Low Copy Number (LCN) Analysis

This technique was developed in 1999 by the UK Forensic Science Service. It was developed in order to increase the sensitivity of DNA analysis methods used in the case of forensic science. Low copy number refers to the process of analysis of template DNA which in amount is less than 200 pg in a sample. In case of standard techniques, the PCR cycles are only kept up to 28, but in case of LCN, for the better quality of results, the cycle's number are increased to 38 PCR cycles [38]. In order to increase better sensitivity to results in case of LCN, various approaches are used in which the amendments are made in case of pre- or post-PCR cycles. Other methods that can help in achieving better results via this method include the use of nested PCR, reducing the volume of the PCR, and the use of pure formamide in case of sample preparation that need to be used in capillary electrophoresis.

There are Various Disadvantages of this Technique as:

- The chances of the error are more as compared to other techniques.
- These profiles generated by LCN are not much reproducible.
- Major problem occurs when different profiles mix during LCN typing; then the results are not reliable.
- In case of LCN, the assay is very sensitive so the samples that need to be analysed require effective handling.
- Some reagents and chemicals used may contain extraneous DNA in very low amounts; these can interpret the results.
- High chances of contamination.

This technique is used for the identification of the cases where biological evidences are compromised and other method for DNA analysis cannot be used. An example of this technique is used in DNA analysis where the results were not reliable due to inappropriate measure used by the technicians in forensic laboratory [38, 39]. In 1998, terrorist attack happened at Ireland in which 200 people severely wounded and 29 people died; the police administration suspected an electrician behind the attack. He was 38 year old. Evidence was collected from the site, and that to sample was collected from the suspect and LCN analysis was done, but unfortunately, the results did not show any matching between the suspect and the evidences collected. At the courtroom, the judge declared that due to the ineffective handling, not using of appropriate measures for handling the evidence and not using precaution while doing LCN analysis, the results are inappropriate [39].

1.4.5 Variable Number Tandem Repeat

In 1985, this technique of DNA analysis was discovered by Alec J. Jaffreys. They are comprising of 7–10 base pairs and are also known as minisatellites. In human genome, either one copy of the variable number tandem repeat locus or multiple copies are present. These VNTRs are inherited from parents to offspring. As per their number, they are divided into two categories, i.e. unique loci and multiple loci [40]. An example of the use of VNTR in forensic DNA analysis is as follows:

Dr. Jeffrey conducted DNA analysis on various cases to know the real culprit. He conducted an analysis by taking samples from Leicestershire area crime scene, along with the police. The saliva and blood samples were collected from 4000 men, but none of the sample matched the evidence collected. Later, a person named Colin Pitchfork confessed that he was paid money to give false sample. After this, he was prisoned, and a sample of him was taken, and DNA analysis was done. The results obtained showed matching of the VNTR to the evidence collected from crime scene. Pitchfork was the first person who was sentenced to jail as he convicted the murder. With the help of VNTR technique, various other cases were solved. In 1987, with the help of this technique, a rape case was solved in the United States. The semen sample collected matched to the traces recovered from the body of the victim [41].

1.4.6 Mitochondrial DNA (mtDNA) Analysis

Mitochondrial DNA is isolated from the mitochondria of the cells. Mitochondrion is also known as the power house of the cells. Mitochondrial DNA is present in a circular form. It comprises of 16,569 base pairs. A minute variation is present between the sequences of two individual as this sequence of base pairs is highly conserved but entirely functional in nature. Out of this 16,569, the 1000 base pair long sequence which is known as the control region consists of non-coding D-loop. The non-coding regions contain hyper variable regions which further undergoes variations [42]. The variations are termed as single nucleotide polymorphic (SNP) regions; they are the main regions of this sequence and are more focussed by the forensic investigators. This technique of mitochondrial DNA analysis is highly effective in forensic investigations in cases where nuclear DNA analysis cannot be done. Such possibility occurs in case DNA damage is high due to either burn samples or hair without root. Moreover, this method is highly used in case of lineage studies as mtDNA is inherited only from mother to offspring, so to form an analysis on lineages, this method can effectively be used. Various steps involved in the process of DNA profiling via the use of mtDNA include primary visual analysis, preparation of the sample followed by DNA extraction and then steps of PCR, i.e. amplification and post-amplification. It is important to carry out PCR step carefully as the area of interest is a hypervariable region [43]. After the PCR, the isolated product is quantified, purified and automated; DNA sequencing and data analysis are done. It is advised to handle the samples carefully starting from the collection to analysis in order to prevent any mixing of the samples and to prevent

cross-contamination between samples. This technique is highly used in case of the missing individual. A reference sample is not available or in case of mass disaster [44].

The first case solved in forensic science by using this methodology was related to a 3-year-old child who disappeared from her own residence. After 2 years of this incident, the remains of a human child were found approximately 3 km away from their residence. From that site, the skeletal remains were collected from the desert. Using techniques of DNA profiling, the control regions of mtDNA were recovered. Those recovered regions were matching to that of her mother mitochondrial DNA [44]. It was observed that mtDNA typing can be used in case of missing individual cases as well as in sexual assault cases.

1.4.7 Single-Cell DNA Finger Printing

In 1997, another great technique of DNA fingerprinting was developed by Dr. Lan Findlay; along with few of his colleagues, he developed the method of doing DNA profiling from a single cell. They discovered this new approach at the Australian Genome Research Facility. In this method, a single cell is isolated by using the technique of microscopy prior to final analysis, the cells obtained for identification are collected by swabbing the material, and then, with the help of microscope, initially, the identification of the cells is done [45]. This method of DNA analysis is effectively used to solve rape cases as the DNA of the sperm cells is highly conserved and they are compacted in a protein head. Moreover, this technique is quite fast solving cases in hours, thus making it easy to find out the criminal at the same point. Single cells can be obtained from the fingerprints or marks on pens and car keys, but the only limitation to this method is the requirement of the DNA. More than 1 ng of DNA is required which is equal to 200 cells.

1.4.8 Y-Chromosome Analysis

In this technique of DNA analysis, the more focus is given on the different types of marker, i.e. amelogenin marker. These markers are only present on the sex chromosomes. A specific part of the Y chromosome of the males is used in forensic DNA analysis. This technique is widely used in case of paternity disputes of male child, in case of sexual assault and traces the donors of the missing persons. Various new systems have been developed in order to analyse the short tandem repeats present on the Y chromosome; one such system is applied biosystems [46]. Y-DNA analysis involves the analysis of short tandem repeat segments on Y chromosome. These STRs are first recognised as genetic markers. These repetitions vary from person to person, and STR present on the Y chromosome contains a unique DYS number. In case of this method, the test usually involves the examination and analysis of the 10–100 short tandem repeats that are present on the Y chromosome.

The sample from the crime scene and suspect are isolated and looked for these repeated sequences.

1.4.9 Single Nucleotide Polymorphism

In cases where DNA is badly degraded, the technique of DNA analysis used is Single nucleotide polymorphism (SNPs). Single nucleotide polymorphisms are present in abundance in human genome. They particularly have low rate of mutation, and the size of the amplicon is also small. Basically, SNPs are caused due to point mutations. They are present in the noncoding regions of the genome sequence. For the sequencing under this technique, some basic steps involved include the development and identification of SNP with the use of shotgun sequencing, PCR amplicon targeted sequencing and RNA sequencing. The short fragments can be amplified by using SNP technique for DNA analysis in forensic science; thus, this make easy to solve the cases where DNA sample is degraded or low quantity of DNA template is available. Moreover, due to their low mutation rate, they are regarded as more stable in nature so they are effectively used in the reconstruction of the pedigree and lineage. They can also be used in the identification of the individuals and phenotypic inference studies [47].

In the Case of Forensic DNA Analysis, These SNP are Divided into Four Categories:

1. **Identity testing single nucleotide polymorphism**—in case of individualization where low inbreeding coefficient and high heterozygosity is required. They provide the genetic information in order to distinguish between the different individuals and also help to exclude those suspects or samples that are not part of the putative family member [47].
2. **Lineage informative single nucleotide polymorphism**—in this, the markers are used for the identification of the missing persons through the process of the kinship analysis. Tightly linked SNPs are used that function as haplotype markers (47, 48).
3. **Phenotype informative single nucleotide polymorphism**—they are used to establish the high link of the probability regarding the phenotypic characteristics of an individual such as skin, hair and eye colour as an investigation [48].
4. **Ancestry informative single nucleotide polymorphism**—they are used to establish the high link of the probability regarding the biogeographically characteristics of an individual in link to the phenotypic relationships [48].

Single nucleotide polymorphisms (SNPs) have great advantages in forensic DNA analyses because of the presence of abundant potential markers and amenability to automation. In addition to this, they can be used for the phenotypic identification of the suspect as the physical description of a person can help to portray the individual, thus making it easy for the bureaucrats to solve some cases. Despite of all these advantages, some limitations are that SNPs are biallelic in nature so they are less

informative for the identification testing as compared to the other methods such as STR [49]. Moreover, some ethical and legal concerns arise in the use of single nucleotide polymorphism because the noncoding DNA regions are used and some rules and regulations are set by the higher authorities that need to be followed as the privacy is the main concern in any case of the DNA profiling. Gene-gene interaction acts as a hurdle for solving the cases of phenotypic informative SNPs.

1.5 Challenges in Forensic Science for DNA Profiling

- Various challenges are faced by the investigators at the site or even with the direct contact with victim as in some cases like rape or sexual assault when the victim is not ready to give evidences or even sample for the DNA profiling to match with the suspected person. This could be due to family or social issues that some people do not want to disclose the right information; thus, it's a challenge in such cases to catch the culprit [50].
- The sample collection needs a great focus as in some cases when the requirement is of blood sample but the authorities related to case fail to provide it and as in some cases the law enforcement people give a number of items collected from the crime scene to the forensic laboratory to solve the case while in actual the requirement is not so. It requires a critical thinking to choose the item which should be sent for further investigation [51].
- Chances of error are high in case of handling the sample of DNA. Sometimes the sample is collected from more than two to three individuals. This can even lead to mixing of samples. Mixing of samples led to challenge for analysis for the DNA in order to produce desired results [52].
- Requirement of developed new bioinformatics tools in laboratory for the handling of large number of samples in case of mass disaster. In such cases, there is requirement of handling, managing and analysis of the collected samples in a huge number; thus, the trained technical staff is required. Moreover, the laboratories are not prepared to handle the complex mixtures [50, 51].
- Conflict of interest between the bureaucrats and forensic investigators as they tend to look more in the history but the scientist shows their interest towards the future [51].
- Sometimes, even the bureaucrats do not want to disclose the right criminal due to social conflicts and corrupt officers, so they try to misinterpret with the data in order to hide the truth, and there is a possibility that DNA can be replaced by the non-criminals sample even at the crime scene [50].
- In case of DNA analysis for solving criminal cases, the degradation of the samples is the major issue which occurs due to mishandling of samples and inappropriate labelling issues. DNA degradation starts with contact in sunlight or heat so proper handling by technicians is required [51].
- In order to mislead the investigators, sometimes, the culprits try to use synthetic or fake marks of DNA. They left fake marks of DNA at the site which creates complications to analyse the different sample. One such case was reported in

1922; John Schneeberger who was a Canadian physician uses fake DNA sample. He raped one of his patients and left someone else semen sample, so at the time of investigation, the blood sample collected from John and the semen collected did not match; thus, it led to confusion among the investigators to solve the case [52].

- Trouble with various instruments that are used, old instruments and biological contamination of the tools led to unreliable results.
- Hacking of the DNA databases is the main concern. The DNA databases comprises of all the information regarding DNA profiles; thus, hacking by the past culprits with the use of technological innovations is the main cause and the challenge that the forensic sciences has to face.
- Various ethical norms act as a major hurdle in case of solving sensitive cases like rape or acid attacks as the privacy of the individual with regard to community and religion affects the process of solving cases. Sometimes people are unwilling to disclose what actually happen. Such scenarios act as a challenge for forensic science investigators for solving a case. Moreover, the privacy concern is related to DNA because it contains a lot of information of an individual such as family relationships and diseases related to a person [51].
- Another major challenges faced by the investigators is at the times when the damage to the body is more, in cases related to burn, sometimes, the criminal in order to hide any evidences burns the body of the victim or the surrounding area; this led to great challenge for the extraction of the DNA from such sites [50].
- A large number of cases are solved per day globally using various techniques of DNA profiling, so numerous data is stored regarding profiles of the DNA of either suspect or victim. For the storage of this data, an expansion of the DNA databases will lead to overburden of crime laboratories which is another major challenge that requires scientific experts to maintain the privacy of this data in order to prevent it from hacking [52] (Fig. 1.5).

1.6 Cases Resolved Using Various Techniques of DNA Analysis in Forensic Science

1.6.1 Case 1

A married couple was found dead in the city of Kicevo. Their bodies were corded and hanged. The samples were collected from the crime scene; blood was extracted from the body of the victims, and the nail debris was taken into consideration as a sample. The collected samples were transported to the forensic laboratory for the process of the DNA analysis. Another sample from rope was isolated as suspect to find actual criminal. The method of DNA profiling was continued with extraction of DNA and amplification of it, and with STR typing, the DNA profile was generated which was sent to forensic DNA database of Macedonian. It was observed that the results showed matching of DNA profile to an unknown male to that of sample collected from the legs of male victim. At the same time, another case was reported of burglary in church. During the investigation, they found bloodstains near the

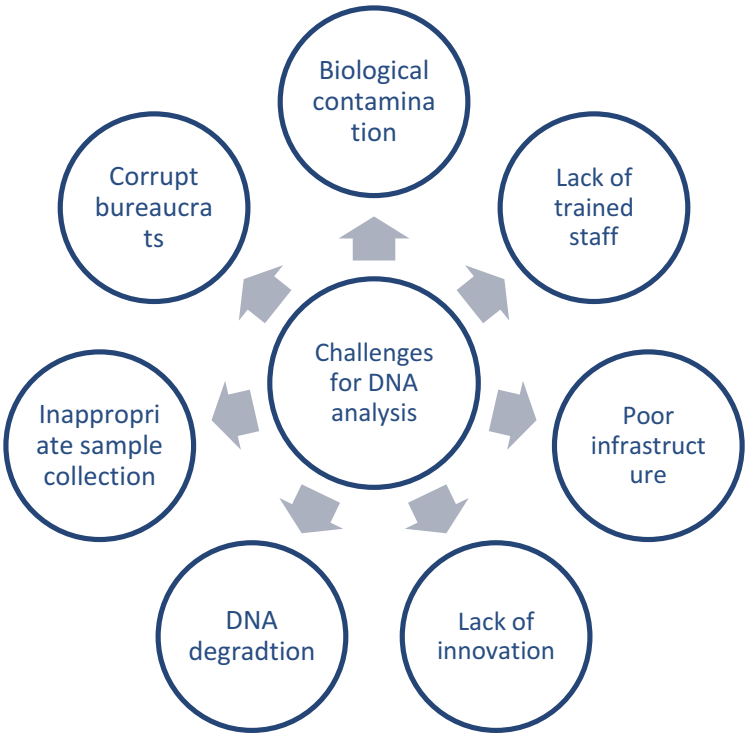

Fig. 1.5 Challenges in forensic DNA analysis

window of the church. The sample was collected from the suspected person, and the DNA analysis was done. The results matched to that of the suspect. The same DNA profile of the culprit was used to run analysis with earlier two profiles of couple murder, and it showed positive results [53]. Thus, the same person was responsible for the murder of married couple and the burglary of the church. This was concluded from the reports analysed by performing DNA analysis.

1.6.2 Case 2

Another case was solved using DNA analysis techniques in forensic sciences. This case was reported regarding the murder of a young man of 35 years old. He was found shot dead in his backyard. Policemen and a team from forensic science reached the crime scene, and as per the witness, several men were observed at the scene. Just after the death, all left. The team started their research and collected three evidences: a handgun, pair of gloves and sleeves of two shirts. The DNA was isolated from the collected evidences, and by using VNTR method, the case was resolved [54]. The results showed matching of profile to an unknown person; this profile was then matched with the DNA data profile stored database of CODIS. The

hit was matched, and shooter was sentenced to in prison for 65 years for murder and robbery.

1.6.3 Case 3

In Bronx, by using low copy number, a case was solved which was related to a gunshot. In 2008, a guy (victim) and his brother had some argument with a group of teenagers. By one of the member of that group, the victim died who was shot with a gun by two bullets. Days after the defendant went to New York, later, he was arrested there as a gun was found in the cavity of the wall in his apartment. From the evidence of the video shoot, an eyewitness to bullet shot case identified that the same man as the shooter. The sample was collected from the person and sent to forensic laboratory to conduct and matched the evidences collected from earlier crime scene. With the technique of low copy number, it was clarified that he was the shooter [53]. He was sentenced to jail for 20 years for the case against weapon charge and manslaughter.

1.6.4 Case 4

A female body was found degraded in an open field during the month of July in 1990. The identification of the body was near to impossible for analysis by individual clothes and fingerprints. The investigators collected fragments of some bones, specifically heel bone and fibula. The samples collected were sent to forensic laboratory for the further DNA isolation and analysis. They tried to amplify the hyper variable regions within HV1 and HV2 present in mitochondrial DNA. On the other side, blood sample was collected from the putative sister of deceased. After analysis, the profile showed matched sequence between the sample collected from decomposed body and her sister. The technique of mtDNA of DNA profiling was used to solve this case as the mtDNA methodology is highly effective to provide reliable results in case of totally decomposed bodies and missing cases [54].

1.6.5 Case 5

In 2012, from the last known place of King Richard III in Leicester, a skeleton was found at Grey Friars. In order to find out if the remains excavated belong to that of King Richard III, the sample was collected in the form of bones, and sequencing was done. The HV1 and HV2, the two hyper variable regions of the mtDNA, were sequenced from the collected sample and collected. This sequence was compared with the sequence obtained from the sample collected from living relatives of King Richard III. The results showed perfect match between the two sequences, and thus, it was confirmed that the remains are of King Richard III. The technique of mtDNA

was widely used for the DNA profiling in cases where mass graves were found in order to determine their lineages to human [53].

1.6.6 Case 6

A 16-year-old girl's body was found in a dense forest on July 6, 2017. She went to school on 4 July and didn't come back, so her parents filed an FIR in nearby police station. Later, her body was found. The case was handed over to Central Bureau of Investigation as numerous marks were found over her body; the team of CBI examined the case and found that the little girl was gang-raped and murdered. The samples from the crime scene were collected which included blood and semen samples; for more evidence, a bottle of liquor and clay were also collected from the crime scene. Later, from approximately 250 people from nearby areas, the blood sample was taken in order to match to the DNA isolated from semen sample, but initially, the results came negative. A percentage and lineage test was conducted by the investigators, and fortunately, they found some match which was with a family from Kangra. Then the samples were collected from that family member to carry out further research and find the true victim. After narrowing down their analysis, they found a person named Anil whose sample was showing matched to that of the DNA sample collected from the crime scene. Anil was arrested by the police, and from the details given by him, the other five suspects were also arrested [53]. By using DNA profiling technique, their samples showed match to that of the semen sample collected from the site. Thus, the case was solved, and culprits were punished.

1.6.7 Case 7

In 1995, a murder case was reported in Utah. A female named Beslanowitch was 17 years old and found dead near Provo River. Her skull was crushed with a hard stone which was later collected from the crime scene for investigation. At that time, the techniques of DNA analysis for forensic science were not fully developed, but the officer investigating this case was curious and dedicated to know what has actually happened. So, later in 2013, when new DNA technologies were introduced, he decided to forward the case to forensic team along with the stone collected from crime scene [54]. By using the technique of touch DNA and with the help of forensic vacuum for the extraction of the DNA, an analysis was made. From the results obtained, the DNA matched to a bus driver who was working in 1995 in a resort near to crime scene. The criminal was arrested and sent to prison.

1.6.8 Case 8

A lady reported a case that she was raped and filed a case against the person to be responsible father of her child. The police started the investigation, and they decided

to carry out the method of DNA fingerprinting to solve this parental issue case. The blood samples were collected from the women, child, and the person he filed a case against. Following the initial step of the isolation, the DNA was extracted by organic extraction method. Further, the quantification of the DNA was done. In order to increase the quantity of the DNA sample, PCR technique was used for the amplification, and at last, the sequence was generated. With the help of the STR technique of the DNA fingerprinting, a 16 loci STR sequence was generated and analysed [53]. The results obtained were quite shocking as the alleles obtained showed matching of the maternal alleles to that of the child, but the suspected person was not showing any matching of alleles, so he was not supposed to be the biological father of the child. Thus, the techniques of DNA profiling in forensic science help to secure the innocent suspects and to punish the real culprits.

1.6.9 Case 9

In woody mountains of the Italy, two corpses covered with very thick vegetation were found. The investigation on them was carried out by the Bresic Forensic Institute; they collected the remains from the area and carried out various investigations on it to determine their age, sex and morphological characteristics from the collected samples. The necroscopic investigators concluded that both were males and their cause of the death was injuries due to gunshot or stabbing. In addition to it, by using mtDNA technology, they were able to analyse the DNA profiles which later showed matched to the three suspects that were arrested for this crime [53].

1.7 Applications of DNA Analysis in Forensic Science

- **Generation of DNA data banks**—Presently, the technique of the DNA fingerprinting is widely used in forensic science for solving various cases, and a lot of DNA is isolated to generate a sequence. Each individual has a specific sequence, and each sequence plays an important role for generating a DNA database. In order to preserve the DNA fingerprints of all the collected samples on daily basis, the Federal Bureau of Investigation (FBI) has created data banks [55]. These data banks are maintained and handled by potential team of expertise, thus providing a number of resources to the people working and maintaining them. Moreover, these databanks are maintained in order to solve the criminal or any other cases by the use of techniques of DNA fingerprinting.
- **Paternity determination**—The technique of the DNA analysis plays an important role to solve the paternity dispute cases of various offsprings. Moreover, it can also be used to identify the dead person. In the cases in which the bodies of the victim are completely burnt, the bones can be used to determine the DNA of the body in order to find out the true identity of the person. Earlier, the ABO blood typing was used, but now with the advancement in science and development of

the new technologies, we can determine the DNA of the offspring and match to their respective parents to solve parental issues [55]. ABO blood typing is not used as the potential technique to solve such cases as the blood is inherited to child on the basis on dominancy in character, so it is preferred to go for DNA fingerprinting to solve such cases. 99% of accuracy is there in DNA typing. The blood samples from the child and parents are collected, and sequencing is done. The matching for the strands of the two samples determine the actual parents of the offspring. A case was solved by using DNA profiling, in which a man was doubting that out of the three of his sons, he is not the biological father of the first one, so he approached the forensic lab and as per their advice he collected hair sample from the comb and sent that to lab. Later on, the results obtained showed the matching of DNA profile of all three to him; thus, his doubt on his wife was wrong [56]. This is how the method of DNA profiling is successfully used to solve paternity cases, and it is widely accepted on legal basis.

- **Identification of rapists**—Various cases have been solved with the help of DNA profiling. Currently, a rape case can easily be solved with no time. Samples can be collected by the technicians either through vaginal swab or through semen found at the site of the crime. Furthermore, the geneticist can separate the sperm cells from that of women cells by various techniques to determine the DNA of the man from the semen sample. Firstly, the purification of the sperms is done with the help of the restriction enzyme. Following it, fingerprints are generated which are further compared to the blood collected from the suspect as well as to that of the evidence collected from the crime scene [57]. For example, a gang-rape case was solved in 2007; a girl was found dead in a dense forest. The samples as evidence were collected which included a bottle of liquor and stains of blood and semen. Later, by using the method of lineage and percentage test, the CBI was able to found out the culprit Anil who was hiding far from the place of the crime. From the sample collected, DNA profiles were made and then were compared to the profiles of the other four suspects along with Anil. The results showed match, and they were punished for their crime [56]. Thus, the techniques of DNA analysis play an important role to identify the rapists easily.
- **Identification of human remains**—It is a very complex process to examine the remains of an individual excavated from a site, but with the advancements in science of the forensics, one can identify the remains of a person. It can be done by collecting the samples such as bones and then isolation of the DNA from it, further analysing the DNA profiles in order to find out their relationship with those alive [58]. A very careful analysis needs to be done with no hope as sometimes the corpse found is not in condition even to isolate anything. Various cases have been solved by using this approach. One such is regarding the remains of the King Richard III of Leicester. His skeleton was found at Grey Friars. In order to find out if the remains excavated belong to that of King Richard III, the sample was collected in form of bones and sequencing was done. The HV1 and HV2, the two hyper variable regions of the mtDNA, were sequenced from the collected sample and collected. This sequence was compared with the sequence obtained from the sample collected from living relatives of King Richard III. The

results showed perfect match between the two sequences, and thus, it was confirmed that the remains are of King Richard III. The technique of mtDNA was widely used for the DNA profiling in cases where mass graves were found in order to determine their lineages to human [57, 58].

- **Identification of murderers**—The science of forensics has a broad spectrum of applications in the field of solving various criminal cases, and the results are accepted by the legal system because the scientific protocols are used to solve the cases. Various steps are involved for the identification of a murderer by DNA profiling starting from the recognition to the evaluation of the evidences collected from the crime sites. The collected samples are sent to the forensic labs for the analysis of the DNA profiles of both the victim and suspect in order to find out the true relationship between them [59]. Various factors are involved for the introduction of the forensic science to the identification of crimes some of them are societal vagaries and obscurity, with all the advancements in science and technology where a number of positive aspects are there but negative impact led to the involvement of forensics of the field of crime solving branch. Globally, a number of cases related to murder have been solved by using the techniques of the DNA analysis; for example, a case in Delhi was solved by CBI in which a housewife was murdered by her own best friend as she was having relationship with her husband. In this case, the women was found lying on the floor flooded with blood, a knife was found in the dustbin of their kitchen, and few scratches of skin were isolated from the body of the victim. Later when sent for investigation from the skin samples, isolated DNA profile was generated. The samples were collected from all the close family and friends. Later the match of profile was observed with her own best friend, and the case was solved [58].
- **Epigenetics**—It is another emerging field of science that plays a great role in the forensic field. This field focuses on the study of the non-heritable changes. Basically, epigenetics involves the alterations of the DNA that does not affect the sequence of the DNA, but the activity of the DNA is affected. The main focus is given to the aging and diseases in epigenetics. In addition to this, the DNA methylation profiling is regarded as one of the most important techniques used for the investigation purposes [60]. It is used for the identification of the tissues and the aging process. Epigenetics is an application of DNA profiling that is widely used in forensic sciences.
- **Analysis of non-human DNA**—In some cases, it has been observed that the bodies are found in forest. The condition of them is worst to conduct a study and analysis on them in order to achieve the target results. In such scenarios, it is important to find out if the DNA isolated from the sample is human or non-human as various microbes are present on decomposed bodies [61]. The type of DNA, either human or non-human, can be determined by using genome profiling technique in which patterns are obtained on electrophoresed gels using multiple DNA samples. But only with the help of a single primer, the diagnosis is done. The advantage of using genome profiling is that in a very short time span, the results can be obtained; moreover, limited technical skills are required**.** In addition to this, real-time PCR technique of the DNA analysis can also be used for the

detection of the nuclear gene target which is specific in nature. A foxhead box (FOXP2) can be used to distinguish between the kinds of DNA. It is more specifically either human or non-human DNA. Use of FOXP2 is taken as a quantification method. The application of the short tandem repeat can easily determine the presence of non-human DNA; in addition to this, single nucleotide polymorphism and the chip technology can be used for the identification of the distinct species in case further analysis is required during the investigation of the samples collected from the scene of the crime [62].

- **Drug sourcing**—In cases related to the narcoterrorism, it is important to figure out the origin of various drugs. The method to determine the source of the various drugs such as opium and heroin is known as drug sourcing. The methods of the DNA fingerprinting such as mtDNA markers and chloroplast DNA are effectively used to identify the geographical origin of the drugs with the help of the mutlilocus system that is used for the prediction [63]. In various countries like Brazil, STR multiplex system was used to create genotype of marijuana samples. A case related to drug transportation and murderer was solved with the help of the DNA profiling method where the drug dealer was arrested later on the basis of the results obtained.

1.8 Nanotechnology in DNA Analysis

Nanotechnology is the branch of the science that deals with the nanoparticles in the field of the research and development. The word 'nano' is a Greek word which means dwarf. The size of a nano is 40,000 times smaller than the width of the human hair; in other words, a virus which is 100 nm in size describes the size of a nano. The use of nanoparticles in forensics helps to solve the cases easily; mainly, it helps to reveal the hidden evidences from the samples collected at the crime scene. Forensics is a wide branch under which the use of the nanoparticles is used for the several investigations such as fingerprinting development, drug identification and manufacture of biosensors [64]. Some of the applications of nanotechnology in forensic DNA analysis are discussed as follows:

- **In-depth diagnosis**—The field of the nanotechnology helps to solve the various cases from the evidence collected; the samples are diagnosed and examined at very nanoscale level. In cases of samples in which the heavy metals and gunshots are there, the DNA fingerprinting can be done with the help of the nanotechnology [65]. By using the approaches of the nanotechnology, the samples can be handled at the nanometre scale, and in-depth analysis of the cases can be done.
- **Amplification of DNA**—The samples for the DNA extracted from the skeletal and body fluids can be amplified in high quality; with the help of the polymerase chain reactions for these, different types of the nanoparticles are used such as magnetic and copper nanoparticles that involve the use of the silica in forensic analysis. For instance, in the process of the PCR amplification, the use of the nanoparticles is introduced due to their ability of uniqueness in different physical

and chemical properties. Gold nanoparticles (AuNPs) are used to increase the specificity of the polymerase chain reaction. In addition to the gold nanoparticles, the carbon nanotubes and the silver nanoparticles are also used to enhance the specificity of the PCR [66].

- **Biosensors based on nanoparticles**—Nanotechnology has a great ability to create and manipulate devices; thus, it is widely used for the production of the kits used in the forensic laboratories for the determination purposes. Various chip-based technologies can be used for the analysis of the DNA in a very short time [66]. A vast variety of applications are there of nanotechnology such as electric engineering, biomedical science and material sciences due to which the nanoparticles can be utilized in every field of the science.
- **Drug detection**—An important role of the nanotechnology is in the identification as well as the examination of the toxic substances isolated from the critical samples such as hair, blood, saliva and fingerprints collected from the crime scene. One such example was quantification of the illegal drug cocaine with the help of the titanium nanoparticle. The cases related to sexual assault, robbery and physical harm are solved via this method. In such cases, the psychotropic substances are given to the victims which can be analysed via nanotechnology [67]. Various biosensors have been developed which are utilized on in field analysis of the detection of drugs. New nanosensors are used to detect the drug clonazepam from the samples of the blood and skeletal with the help of the melamine modified nanoparticles of gold. Detection of a narcotic drug such as a codeine sulphate can be done using a mobile phone; this new way of investigation of various drugs at the crime scene is widely practised.
- **Fingerprint identification**—Nanocomposites are used in combination to the hybrid calcium sulphide quantum dots in order to generate fluorescent in the nature; this fluorescent helps in the better analysis of the fingerprints of the DNA present on a surface, and hence, nanoparticles can be used to detect surface DNA prints. It has been observed that curcumin-based nanoparticles extracted from the turmeric are very selective fluorescent probes used in trinitrotoluene detection even in an aqueous solution [68]. Moreover, in order to enhance the clarity of the fingerprints, the integration of the nanotechnology plays an effective role. The use of the gold nanoparticles along with their binding to the long hydrocarbon chains helps to enhance the quality of the fingerprints; thus, it clearly depicts them for further analysis. The latent fingerprints that are present on the multicoloured can be visualised easily with the help of the nano powder [69].
- **Explosive residue detection**—For the investigation in which the detection of the trace amounts of the explosive components is needed to be done, the nanoparticles can be widely used. The detection of the toxic elements can be done from the tissues, contaminated samples and surfaces with the help of wide varieties of nanoparticles for example; in blasts, the investigation is a challenging process as every leftover remains in the form of the fragments [66]. With the help of nano-based sensors and analysers, the investigators can detect microscopic particles with the help of the powder of the gun in which high resolution scanning microscope is used. Moreover, in cases of use of illegal drugs where an accused

handling explosive substances can be caught as in his fingerprints, the traces of the drugs can be found easily with the help of the nano-engineered powder [67].

- **Extraction of DNA**—The nanoparticles help in the extraction and the amplification of the DNA. Nanoparticles can be used to quantify the DNA samples that are used in the forensic studies such as mitochondrial DNA, which can be quantified in a very short span of time. In case where the samples of the DNA are in small quantities, they can be quantified with gold and zinc nanoparticles [65]. For example, the sample of the urine is source of DNA isolation. With the help of the genomic DNA isolation, methods of filtration and centrifugation along with the help of the nanoparticles of carboxylated magnetic, the DNA can be isolated on a solid phase adsorbent. Further, the intact isolated DNA can be amplified.
- **Ion beam analysis**—It is one of the most widely used techniques used in the investigation in case of the forensic science in order to examine the material collected in the form of the evidence from the crime sites. It is used to detect, identify and analyse the material or residues that are extracted from the samples of the gunshot and soil, thus helping in the determination of the elemental characteristics of the collected material [66]. This technique uses the millielectron volts to determine the thickness, nature as well as the position of the elements. Thus, with the help of the X-ray and gamma rays, in addition to the nanoparticles, it can help to analyse various samples collected from the crime scene as per the requirements of the case.
- **Nano-trackers**—In order to combat the crime, various preventive measures are used in the form of patterns on the products that are kept secret from the suspects or public so as to prevent theft; for this purpose, barcodes are used. In case of the forensic science, the nanoparticles are injected into the body of the prisoners so as to track them via nano-trackers that are injected in their body. In addition to this, cases of burglary can also be investigated by using nanotechnology [67].
- **Estimation of time since death (TSD)**—The estimation of time since death plays a crucial role for solving any criminal case specially related to murders as the time estimation helps to know the accurate time and cause of the death. Earlier, the forensic investigators used to estimate the time via physical examination of the body they used to make an analysis on the basis of the algor mortis, changes in the colour of the eyes, changes in the decomposition of the body and the contents from bowel or urinary bladder. With the advancements in science and technology, now the investigators give more focus towards chip method for the analysis of various amino acids as well as determination of biochemical changes [68]. In the forthcoming years, it is expected that with the help of fluorescent nanoparticles, the estimation of the time of death can be done in which the nanoparticle will be used for the quantification of the various body fluids, thus making it easy and time-consuming for solving criminal cases. The actual time of death is very important to solve critical cases as the more time is given to the culprit, the more delays in the process of case solving will took; thus, in every murder-related case, the first thing the investigators tend to do is the determination of the time since death and use of nanoparticles are making it easy [69] (Fig. 1.6).

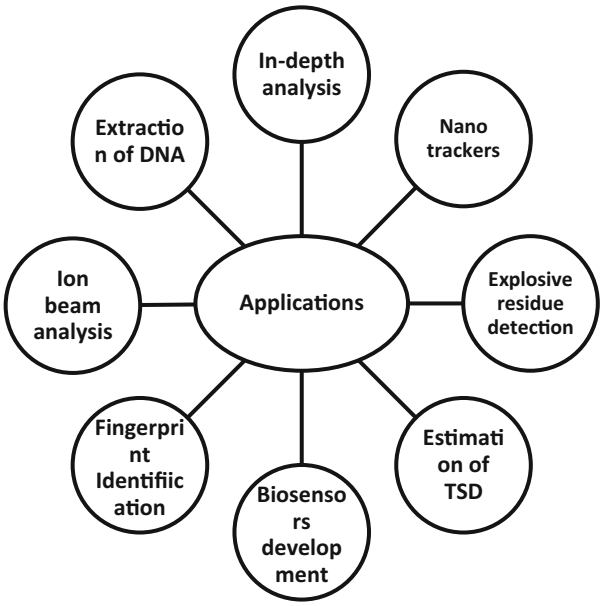

Fig. 1.6 Various applications of nanoparticles in forensic investigation

1.9 Future Advancements of Nanotechnology in the Field of Forensic Science

1. Development of the new advanced media which can be used for the multimodal imaging in solving cases [70].
2. Designing of the nano-structured materials.
3. Conversion of the bioenergy [70].
4. Analysis of the thin films for the biomedical purposes—this will help to do in-depth analysis of the various samples collected from the crime scene [71].
5. Manufacture of the molecularly imprinted polymers in the form of the nanoparticles. These particles can be used for the nanosurface analytical applications [70].
6. Characterization of the functional materials for the investigations on the structure and vibrational properties of the various materials via studying their physico-chemical properties [71].
7. Enhancement in the field of the nano-engineering [70].
8. Introduction of nanotechnology in the field of science for researchers with better tools and equipments in order to increase the number of the skilled workers under this field to make the processes more efficient [71].
9. Awareness for international links with other scientists working on nanotechnology can bring about better advancements for future [70].

1.10 Collection of DNA Samples and Issues Related to Sampling

The first step in investigation of any crime scene by the forensic team is collection of the evidences as much as they can. This step requires analytical and logical skills as the evidences play an important role to solve the case. The samples are collected by the expertise of the team and are preserved for further testing in the forensic laboratory. Samples can be in the form of blood marks and bloodstains, hair, skin, nails, urine stains, scraps from finger nails, teeth and bones in case of burnt cases and different types of touched objects such as cloths, table, vehicle parts, glass, bottles and cosmetic [72]. So, the collection of them should be done separately, and clear labelling is required to distinguish each sample from other. The method of the collection of samples should be clean and precise for example firstly. Identification of the type of sample should be done; then the procedure of collecting it should initiated. But various issues are related with the DNA samples. Sometimes, the sample is minute; due to heat, it may get degraded. Chances of contamination are high. Mixing of the samples and various ethical issues are related [73].

1.11 Methods of Sample Collection

1. **Use of Swabs**—In this method, the first step is creating a defined hole with swab either dry or wet, the co-extraction using multiple swabs is recommended as it enhances the retrieval of the DNA easily. Sodium dodecyl is recommended to be used in case of moist swabs [74]. The yield of the DNA is more from the moisten swabs so after collecting the sample the swab should be immediately frozen to carry out the further processing in the laboratory.
2. **Micro dissection**—This technique of the sampling is used in order to isolate the samples in which the focus is on the specific target cells; with the help of the fluorescence nanoparticles, the labelling of the samples can be done in order to recognize their distinct morphological characters [75].
3. **Laser microdissection**—It helps to distinguish between the cells from the male sample to the female cells, thus making the analysis easy at the first end of the sampling. It is advised to use this method at the time of the sample collection so that the initial labelling between distinct samples can be done [76].

1.12 Issues Related to Sampling

1. The minimum requirement of the DNA sample in order to carry out analysis is 0.1–0.5 ng approximately, but in some cases, the samples are highly regarded, so it is advised to do extraction of the DNA careful in order to prevent any further loss of the sample [77]. The use of organic solvents is recommended while doing the extraction of the DNA.
2. In cases of bomb blast were the number of victims is high and the crime scene is highly scattered, the chances of the mixing of the DNA samples is more due to

poor labelling of different samples collected or due to inappropriate measures used during sampling [78].

3. When the bureaucrats try to collect samples rather than reporting the expertise team in order to save time in such cases they do not use preventive measure and techniques for the collection of the samples, thus resulting in cross-contaminating it with other samples [77]. This makes it difficult for the forensic lab analysts to analyse the sample clearly, so the chance of false results are more. It is advice to report the forensic team at any crime scene in order to collect the samples without any contamination.
4. Degradation of the samples is the main cause to false results or delay in the processing time of any criminal case. The degradation of the DNA occurs at high and humid temperature. It is important to isolate any sample collected from the crime scene in a cool preserving container, and with proper handling, it should be submitted to the forensic lab for further investigation [79]. In addition to this, various environmental issues led to the growth of the bacteria, mosses and lichens on the bodies of the individual are found that led to dilemma at the time of isolation of the DNA whether it is human or non-human DNA.
5. DNA can be transmitted from saliva or when touched by skin directly; thus, it gets contaminated in such cases. Use of the diamond dye chemical is done for sample collection of the evidence, but this process is very cost-effective. In order to prevent such issues, use of cotton or nylon swab is best as nylon swabs or moistened cotton swabs yield high amount of DNA and chance of contamination are less with them [78].
6. The major issues that arises to collect any sample from the crime is the permission from the higher authorities under which the case is reported. It is important to get prior permission from the team to carry out the process of sampling so as to prevent any loss of evidence from the crime site by theft in order to hide the real culprit [77].
7. The storage of samples collected is the main issue as this requires proper security so that not even the family members can access or manipulate anything at the crime scene. Moreover, a large space is required to maintain samples for longer duration [79].

1.13 Forensic Examination Levels

Whenever a case is registered, it is important to recognize the need of various forensic tools for its examination; therefore, the knowledge of tools and services gives an idea to the investigator about different evidences he/she can seize to carry out their investigation. Starting from the physical examination, fingerprint matching, hair and fibre analysis to forensic pathology, anthropology and entomology, various stages of analysis are covered in order to generate the final end report with accurate results [80]. Some of the tools used for examination prior to DNA analysis step in forensic investigation are discussed as follows:

1. **Physical examination—**Different types of evidences can be found during the physical examination of the crime scene that might include marks on tools, footprints indicating a path followed by accused, tire print, torn edges of material like paper, tape or cloth, stains of blood and saliva or some pieces of valuable products that act as a major source of evidence in solving a case. Under physical examination, there are two levels, level one in which the characteristic of the class in relation to the items is taken into consideration such as impressions on tolls, shoe and tire prints. The classification focuses on the model, size and pattern of the various designs, for instance, the size of the foot print any brand if they can determine or pattern [81]. The next, level two, includes the determination of the accidental characteristics such that the investigators look for the items like broken glass and plastic fragments. The fragments of the broken glass can help to determine the direction of a bullet passed, thus making it easy for the investigators in cases of gunshot and murder to establish a link between the origin of shooter and direction travelled by accused.
2. **Fingerprint matching**—This type of examination plays a very important role in forensic science as fingerprints are different from person to person and are representing an unique pattern of lines and ridges that exit on the plantar surface of a person. The identification mark from that of the site of crime can be taken and compared with the database containing patterns of fingerprints stored in it, and matching can be done easily, but now a technique of automated fingerprint identification system is used in which biometric scanning of fingerprints is done electronically [81].
3. **Examination with ballistic approach**—Use of ballistic fingerprinting in forensic helps in examination of the trajectory of the bullet. Trajectory means the path travelled by the bullet from the time it leaves the gun barrel to the end point where gun powder comes to rest. Various patterns of the trajectory are observed by the investigators to draw out the final analysis about the direction of bullet travelled [82].
4. **DNA analysis**—Currently, the laws and regulation set by government are in favour of consideration of the process of DNA fingerprinting as important in forensic analysis because it gives the exact matching of the genome sequencing, thus reducing the chances of error in catching the accused. The process of DNA analysis is very simple, but the collection of the data from the crime sites is important in order to isolate the accurate DNA sequence from the collected sample within a given time frame [82].

1.14 Analysis of Non-human Species Via DNA Profiling

During the analysis of various samples collected from the site of crime, the chances are more of presence of non-human DNA, and distinguishing of a non-human DNA from a human one is very important to determine the results accurately. During forensic investigation, different categories of the biological material is found at the site. It can be in the form of any botanical material; microbial in nature, presence of

dog or cat hair, feathers of birds, saliva, faeces or other animal products can be present. In some cases where bodies are found in damp forest areas, the chances of growth of lichens, bacteria and other microorganisms are more; such organisms generate a challenge for the forensic investigators to isolate any other form of material if present near or on the surface of the body [83]. For instance, in a rape case, if the body of the victim is found in forest, the growth of microbes will interfere with semen or any other sample present near or on surface of the body, as when evidence will be collected the DNA might be of microbe rather than the actual convict; thus, it is important to analyse the sample properly to determine the nature of the DNA isolated. Various methods of genome profiling can be implemented in order to multiply DNA fragments isolated from the sample collected from the site of the crime either by using a random primer or with the help of electrophoresed gel pattern, but these methods sometimes fail to generate reliable results in case of the presence of mixed genomes [84]. For the detection of a human nuclear gene target forehead box P2 (FOXP2), it can encode a transcription factor that accumulates any changes in the amino acids which are involved in human lineage development of the characteristics of language and speech. Thus, FOXP2 can be used to distinguish between a human and non-human DNA. In addition to this, various other methods of DNA analysis can be used such as single nucleotide polymorphism, short tandem repeat, variable number tandem repeat and restriction length polymorphism.

Recently, the technique of the mitochondrial DNA is widely used in the analysis of the non-human DNA due to their salient features such as mitochondria present in almost all eukaryotic cells, and they are termed as powerhouse of the cells as they generate energy that organisms require sustaining livelihood. Mitochondria contain their own genetic information; moreover, they possess remarkable features of the genetic variations such as size, conformation and arrangement of genes [85]. This arrangement of genes is different from person to person or organelle to organelle either in plants, animals or humans. The main advantage of use of mtDNA in forensic DNA analysis lies in their high copy number of the cells. This method is highly used in case of lineage studies as mtDNA is inherited only from mother to offspring so to form an analysis on lineages, this method can effectively be used. Various steps involve in the process of DNA profiling via the use of mtDNA include primary visual analysis, preparation of the sample followed by DNA extraction and then steps of PCR, i.e. amplification and post-amplification. It is important to carry out PCR step carefully as the area of interest is a hypervariable region. After the PCR, the isolated product is quantified, purified and automated. DNA sequencing and data analysis are done [84]. The samples are advised to handle carefully starting from the collection to analysis in order to prevent any mixing of the samples and to prevent cross-contamination between samples. The traceability of food products and endangered species or drugs can be determined with the help of mtDNA technology.

1.15 Future of Forensic DNA Analysis

Various technical developments led to the advancements in the DNA profiling with short time and greater probability of accurate results. The future of the DNA fingerprinting in the field of the forensic will be great with faster, higher and reliable results [85]. With increase in research under this field from past years, focus on the development of more new methodologies has also been increased. The demand of DNA fingerprinting will be high to solve various cases; already with developments, a large amount of DNA can be isolated even from a degraded sample, and much more such advancements are under study.

- A fully automated instrument for the process of DNA profiling has already been introduced. Earlier, the entire process of the DNA sequencing used to take days to solve a case, but now only 90 min are enough for the DNA extraction, amplification via polymerase chain reaction followed by DNA separation and detection, sizing and at last genotyping. With the help of such advancements in short time, the convict can be easily caught as a suspect can be taken in custody for 4–5 h and samples of suspect can be taken, and within a day, the results of DNA sequencing can be achieved [86].
- Sci-Fi—It is a handheld device that can be taken to the crime scene. It is defined as a lab on the chip; the chip would be enough to test the samples at the crime scene in order to generate their sequences of the DNA. This method will provide great advantage as the number of samples can be tested at a single time and place; moreover, it is less time-consuming [87].
- A single integrated platform for the extraction, amplification and sequencing of the DNA has already been developed with the help of microfabrication of capillary electrophoresis, but validation of such techniques is still under the process in order to utilize them freely in forensic sciences for investigation purposes [87].
- Multidisplacement amplification technique can be utilized for the amplification of the whole genome where the amount of the template DNA is quite small [88].
- In case of the identification of body fluid samples, some challenges are faced by the forensic biologists in order to solve this issue. The use of mRNA approach for the identification of the blood, semen and saliva is used in which analysis is made through the specific sequences of mRNA [89].
- With improvements in the PCR assays, more data will be available from various biological samples less than one database; thus, this would provide higher sensitivity towards the information obtained from the sequence analysis of the alleles [90].
- In the coming future, more data will be available in the databases; internationally exchange of data will also benefit to solve old cold cases related to murders and other crime as with exchange of international data more access will be there to more STR loci which will eventually benefit the investigators to solve cases [85].
- The collection of the numerous data from the short tandem repeats (STRs) and single nucleotide polymorphism (SNPs) will serve as a next-generation

sequencing (NGS) approach with the involvement of the parallel sequencing. The information from NGS will require specialized tools of the bioinformatics in order to process the data [85].

- Introduction of phenotypic inferences extracted from a DNA sample so as to compare them to pharmacogenetic information for molecular autopsy. This will also help in determination of the type of tissue and expression analysis [87].
- Some techniques like LCN typing of DNA profiling require new approaches in which the increase in availability of the templates is needed; in addition to this, there is a need of generation of the contamination free reagents that will support LCN analyses with decontamination protocols [86].
- More focus need to be given on the extraction of the DNA as the samples are collected with the help of cotton swabs and from which the DNA is not efficiently removed during extraction, so the new approaches should be developed for efficient extraction of the DNA [85].
- Already various techniques have been developed to isolate the DNA from the degraded samples isolated from the crime scenes, but some PCR inhibitors fail to reproduce the very informative Q profile. For such cases, the smaller PCR amplicons are required. These amplicons can be in the form of the miniSTRs or SNPs [86].
- A better future of the DNA profiling in forensic science can only be seen if the cost for various analyses is reduced. Moreover, technological advancements are needed in laboratories to hold large amount of the data and also to secure the samples for future studies [91].
- The future of any research is dependent on the funding as for the development and research of any new scientific protocols required some sort of funding. Companies or investors only invest in projects if they see any sort of benefits in the forthcoming years so in order to attract the investors to the field of forensic, awareness among people related to various applications and uses of DNA fingerprinting in forensics science is needed [91].
- In the USA, on-site DNA analysis technique has already been introduced; up to some extent, the analysis of various samples can be done on the crime site with the use of various handheld devices [92].
- In future, the use of portable devices for the DNA detection will be more. In comparison to the old PCR techniques, the need is to develop a device that is portable and quantitative and can easily be operated by public. An example of such is a growing demand of the personal glucose meters, i.e. PGMs. These PGMs are used for the quantification of DNA. In addition to this, they can also quantify organic molecules, proteins and metal ions that are linked to the functional molecules of the DNA sensors [90].
- Developments of biosensors that utilize nanoparticles are in demand as nanoparticles such as gold nanoparticles have ability to attract the charge present on the DNA sequence. Thus, various biosensors can be used for the identification of the DNA such as DNA field-effect device (FED); when it is covered by a gold nanoparticle (AuNPs), it attracts the DNA towards itself, thus helping in identification [93].

- An improvement in the statistical analysis of various DNA samples is required in order to attain the reliable results. Moreover, such approaches will lead to improve in selectivity when dealing with the mixture of the DNA samples [85].
- Coupling of the nanotechnology with the microfluidic devices is under research in order to generate high throughput of the DNA profiling [91].
- Morphological analysis of the skull using three-dimensional computer automated techniques is under study in field of the forensic biology. In addition to this, determination of the colour of skin, hair and eyes with the help of the various techniques of gene sequencing is under the next-generation technologies of the DNA fingerprinting [92].
- Use of virtual autopsy that is virtopsy is in the near future in coordination to the forensic biology; in this method, the collection of the images will be done [93].
- Introduction of phenotypic inferences extracted from a DNA sample so as to compare them to pharmacogenetic information for molecular autopsy. This will also help in determination of the type of tissue and expression analysis [93].
- Some techniques like LCN typing of DNA profiling require new approaches in which the increase in availability of the templates is needed; in addition to this, there is a need of generation of the contamination free reagents that will support LCN analyses with decontamination protocols [94].
- More focus is needed to be given on the extraction of the DNA as the samples are collected with the help of cotton swabs and from which the DNA is not efficiently removed during extraction, so the new approaches should be developed for efficient extraction of the DNA [92].

1.16 Summary

The use of deoxyribonucleic acid, i.e. DNA, for the testing in criminal justice explains the term forensic DNA analysis in simple words. It was first introduced in 1981. The term forensis which is a Latin word has given birth to the forensic science where forensic means pertaining to; thus, the term forensic sciences means the use of various applications for the resolution of criminal disputes either criminal or civil. The DNA analysis has become an indispensable part of the modern forensic science; with the use of PCR techniques, it has become a major tool for the analysis of the biological material. Various techniques are discovered for the proper determination of the samples; some of them are low copy number, restriction fragment length polymorphism (RFLP), short tandem repeat (STR), variable number tandem repeat (VNTR) and mitochondrial DNA. The entire process of the DNA analysis is divided into four major parts, namely, serology, which further comprises of collection of sample followed by storage and its characterization. After serology is biology in which the isolation of the DNA is carried out; this involves extraction, quantification, amplification of fragments of the DNA and STR markers. The next step requires the technological aspects in which separation or detection of the DNA is done. After this, interpretation of the data is carried out to determine the characteristic of the isolated DNA from the sample. At last, the role of genetics is there in which

the statistical interpretation is done; this helps in final analysis of the sequence of the DNA isolated from various samples. The genome sequence is compared with that of database DNA or with the suspect DNA extracted so as to find out the actual culprit behind the crime. Various challenges are faced by the investigators at the site or even with the direct contact with victim as in some cases like rape or sexual assault when the victim is not ready to give evidences or even sample for the DNA profiling to match with the suspected person. This could be due to family or social issues that some people do not want to disclose the right information; thus, it's a challenge in such cases to catch the culprit. Currently, the requirement of developed new bioinformatics tools in laboratory for the handling of large number of samples in case of mass disaster is more. In such cases, there is demand for handling, managing and analysis of the collected samples in huge number; thus, the trained technical staff is required. Moreover, the laboratories are not prepared to handle the complex mixtures. Recently, the use of nanoparticles in forensics helps to solve the cases easily; mainly, it helps to reveal the hidden evidences from the samples collected at the crime scene. The nanoparticles help in the extraction and the amplification of the DNA. Nanoparticles can be used to quantify the DNA samples that are used in the forensic studies such that mitochondrial DNA can be quantified in a very short span of time. In case where the samples of the DNA are in small quantities, they can be quantified with gold and zinc nanoparticles. In future, the use of portable devices for the DNA detection will be more in comparison to the old PCR techniques; the need is to develop a device that is portable and quantitative and can easily be operated by public. An example of such is growing demand of the personal glucose meters, i.e. PGMs. These PGMs are used for the quantification of DNA; in addition to this, they can also quantify organic molecules, proteins and metal ions that are linked to the functional molecules of the DNA sensors.

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