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Handbook of DNA Forensic Applications and Interpretation

Springer

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This book is dedicated to Padmashri Dr Lalji Singh, The Father of Indian DNA Fingerprinting.

Dr. J. M. Vyas
Vice Chancellor

FOREWARD

More than 50 Nations across globe now have DNA legislation governing the creation and use of DNA databases. Not only they have technical capacity for testing large numbers of samples grown over the past decade, but also the understanding has increased of the evidentiary power of DNA databases in their ability to combat crime, support human rights, and helping to promote a safer society. Once DNA databasing has been initiated within a nation, the trend is to expand from whom DNA samples are collected and to date there is not an example of legislative changes that resulted in a decrease in types of crimes in which individuals are required to submit a DNA sample. Many states and nations that first passed legislation to collect DNA samples from individuals convicted of the most serious felonies revised their legislation to include a broader range of convictions, with the current trend to collect samples from individuals who have been arrested for certain crimes. This evolving legislation has a direct impact on the DNA laboratories as a change in such legislation can create a sudden surge in the number of samples collected and thus in the desired quantity of DNA testing. The science of DNA testing has continued to evolve by utilizing better robotic systems, improved DNA testing and sequencing equipment, a diversification of DNA testing kits with increased sensitivity, incorporation of laboratory information management systems (LIMS), and an overall increased understanding of the best laboratory procedures. These have combined to increase the number of samples an analyst can process per month and thus the overall throughput of DNA laboratories. Complimenting the increased capacity of DNA databasing is the increasing ability to obtain DNA profiles from crime scene samples. The advent of touch DNA testing has been used to obtain DNA profiles from miniscule amounts of DNA material and such results have been accepted into the court room setting. Such advances are altering the type of cases being received in DNA laboratories and in some cases burglary cases have become one of the most dominate types of cases submitted.

DNA is the source of life and has been studied since a generation, but much more is yet to done to explore various facets of molecular justice. Several sophisticated technologies of the current era have laid their foundations on the principle of DNA based mechanisms. DNA based technologies have revolutionized individual identity helping in scientific investigation, medical diagnostics, paternity dispute resolution, health and motor vehicle insurance etc. Recent years have witnessed an explosive growth in biological data and gave birth to the pacemaker of biology called Bioinformatics. More than 350 fully sequenced genomes are publicly available, and more than 780 are in the pipeline. The expansion of publicly available information resulting from the Human Genome Project has justified the role for bioinformatics capabilities worldwide.

Springer Handbook of "DNA Forensic Applications and Interpretation" is a book aimed to engross the readers for and providing a detailed and cutting-edge level information about DNA technology and its importance in both research and adjudication. The content of this book highlights various DNA based technologies and their profuse applications in several domains of life. Each chapter showcases salient features of DNA and its unique identifier capabilities. It also deals with the context of DNA in several high-end technologies. Few selected thought-provoking case studies have been outlined in these chapters revealing the vital role played by molecular level evidence helping in individual identification with greater precision. A total of 12 chapters are included in the book, each presented by a domain expert with authentic and focused information. The book is an archive of veritable information about applications of molecular techniques in various fields of life. Chapters have been penned authors having specialized field experience, compiling into a very good hand out providing complete spectrum of information to researchers working in molecular biology,

police, prosecution, defense and judiciary for understanding application of the molecular evidence in crime investigation and civil dispute resolution.

Introductory part constitutes the details about the elementary information related to genetic material, the DNA and its importance. Chapters also furnish some crucial case studies explicating the role of DNA in the witness box during crime investigation. Few chapters deliberate brief series of events during the evolution of DNA based forensic investigations. The content further elaborates various procedural protocols for delineating tedious processes involved in DNA sample collection, packaging and labelling and protection of Chain of Custody for regulating the sample integrity and case confidentiality. The chapters also explain various steps involved in processing of the DNA sample after extraction like STR profiling, Fingerprinting, Paternity and maternity testes, DNA Test report generation etc. An additional elaboration is also provided for reporting standards in DNA test.

The application of DNA technology in establishment of individual identity and relationship with the tested individual is also elucidated. Contents expounds some of the sophisticated protocols like RFLP, PCR, STR Profiling etc., which are the inherent parts of DNA based investigation. Additionally, a brief introduction is provided about Forensic Bio-informatics and its applications. Some vital concepts of LIMS, RFID, Biometrics etc., are well explained for the readers and the practitioners. An add on has been provided to address challenges emerged at different stages especially handling difficult samples for extracting DNA. Case studies involving the application of this high-end technology has also been furnished in the book.

The book content also delineates the steps involved in the extraction of DNA from plethora of sources followed by its identification based on band patterns obtained on agarose gel. A special chapter demonstrates the usage of DNA technologies for criminal Adjudication. This is a highly specific and complex briefing of the vital role played by DNA in crime investigation and criminal justice. The chapter details along with important case studies making conceptual clarity for data authenticity and judicial deliberation. Authors being the practitioners of system had direct access to several case reports and it makes the content easy to go for a reader. Legal Contours for DNA Evidence is another chapter which dealt with the challenges faced by Judicial team and technology to establish 'truth', an essential ingredient for justice delivery. A focus has been laid on the scientific standards for DNA evidence in a chapter related to DNA Legislations Challenges and Opportunities. This book also deals with relevant legislations handling DNA technology. A chapter on importance of DNA in individual identification specially in mass disasters is also a significant constituent this book. It further focuses on importance of genetic testing in dead body identification, which is very significant for various socio-legal reasons. The book in hand provides 'One Stop Centre" for information an aspiring reader may be looking for. I have considerable pleasure in observing that the book in hand is a ready reckoner to all actors involved in DNA profiling in different capacities.

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Preface

DNA technology is a high end state of art technology in the field of molecular biology. Its immense applications and authentic data generation capabilities have made it a technology on demand by forensic and legal professionals. This book is not limited to the boundaries of subject and domain as it is an application oriented science with a plethora of implications. The book covers most of the important aspects of DNA based technologies from the basic scratch to the sophisticated applicability in the forensic area. Its contents can be used as a precious handout for Police, Judiciary and legal practitioners and scholars who are interested towards the technical aspects of DNA and its application in their domain of work. All the chapters are presented by prominent individuals in this field. The book is a blend of information related to DNA technology and its applications in the arena of Forensic investigation, Judiciary inspections and resolution of civil and criminal disputes. Being an interdisciplinary handout, it is a great resource for individuals and authorities from the Police department, Forensic institutions, Researchers and Medicolegal professionals. The information furnished in the book includes technical laboratory protocols set along with chapters containing case studies. Role of DNA technology in disaster victim identification is well elaborated in this book. Various technical concepts of RFLP, STR Profiling, DNA Fingerprinting, LIMS, RFID, Biometrics etc, are well explained. The authors of this book are practitioners and researchers from prestigious institutes which includes Indian Police Department, Internationally acclaimed Forensic Professionals, Judiciary making the compilation a great knowledge archive. Some of the chapters in the book are also focused on aspects and challenges faced by technologies in Legislature and decision making. This can resolve the conflicts related to ethical issues of DNA jurisprudence. Overall the compilation can influence individuals from an array of sectors related to criminology and Forensic sciences. This handbook can be advised and utilised for forensic and criminology researchers, police officers, Doctors, lawyers and scholarly intellects.

Hyderabad, India

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Part I
Insights and Advancements in DNA Forensics

Chapter 1
DNA: The Master Molecule

Amit Kumar, Amita Kashyap, and Edwin Huffine

Introduction of DNA

All living creatures have DNA (deoxyribonucleic acid) as their blueprint for life. This DNA primarily exists in the famous double helix and is composed of four bases, adenine (A), cytosine (C), guanine (G), and thymine (T), and these bases form specific pairs with each other on the double helix (A with T, and G with C).

DNA or deoxyribonucleic acid is a genetic material present in the nucleus of cells in all living organisms and DNA is unique for each and every individual just like fingerprints. It is a DNA present in every one of us that makes us distinguishable from each other. Hence DNA is an excellent identification parameter that differs from individual to individual.

DNA analysis deals with DNA fingerprinting of all types of forensic samples of human origin like blood, bloodstains, cement stains, vaginal swabs, tissues, bones, teeth, and other skeletal remains.

Deoxyribonucleic acid or DNA is referred to as our genetic blueprint because it stores the information necessary for passing down genetic attributes to future generations. Residing in every cell of our body with the exception of red blood cells which lack nuclei DNA provides a computer program that determines our physical features and many other attributes. The complete set of instructions for making an Organism the entire DNA in a cell is collectively referred to as its genome.

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Half of the DNA is inherited from a person's mother another half from his or her father. Siblings inherit different combinations of DNA from the same parents and are therefore different from each other. Each generation of people is a new and different combination of genetic material from the previous generation. Except for the identical twins each person's DNA is unique, although the technology available does not yet allow the examination of every single difference between people's DNA.

In total, human DNA has approximately 3 billion bases. Long sequences of these bases give rise to various genes and specific genes code for specific proteins by first being transcribed into messenger RNA (mRNA) and then the mRNA being translated into proteins. Most human DNA does not code for any specific protein and is often referred to as "blank DNA". Just as there is great variety among humans, so there is great variety among the genes, and from this, there is also variety among the DNA sequences of the A, C, G, and T that comprise the genes [1].

Human DNA is divided into 23 pairs of chromosomes, which contain the total of the ~3 billion bases of DNA [2]. Genes that code for certain proteins are always found in the same location on chromosomes among all people. Difference in base-pair sequences within the genes gives rise to alleles, which are different varieties of the same gene. In addition, groups of people whose ancestors originated from various parts of the world have alleles that are found to have originated from that specific location. This has given rise to several ancestry tests where individuals submit their DNA samples to obtain an analysis of their ancestors. Even though the DNA between humans is more than 99% similar, it is these differences that are targeted in forensic investigations. With the exception of identical siblings, everyone's DNA is unique to that person, and there are indications of some variability between identical twins. It is this uniqueness that gives DNA its power to identify.

In humans, there are two sources of DNA: mitochondrial DNA (mtDNA) and nuclear DNA. MtDNA comes from organelles located in the cytoplasm called mitochondria, while nuclear DNA is located in the nucleus of the cell. The mtDNA is much smaller than nuclear DNA and is generally around 16,570 base pairs in size. This DNA codes for the replication and operation of the mitochondria, which are responsible for the production of energy in the cells. Each cell may have dozens of mitochondria and each mitochondrion may have multiple copies of its DNA, meaning there could be hundreds of copies of mtDNA in a cell. MtDNA is maternally inherited, meaning a mother and her children will have the same mtDNA sequence, with the exception of when mutations occur. There are regions in the mtDNA known as hypervariable regions, that do not code for proteins and thus, whose conversation of their sequence is not critical to the functionality of the mitochondria. Therefore, these areas are more prone to mutational events, which give rise to variability in the mtDNA sequence. It is these differences that are used to link a sample to a maternal lineage. As all maternal relatives should have the same mtDNA profile, mtDNA does not point to identity but can be used to help support the linkage of a sample to a particular individual. Also, as there are hundreds of mtDNA sequences per cell, there are times mtDNA may still exist within a cell after nuclear DNA is no longer obtained and testable [3].

Nuclear DNA exists as a pair of 23 chromosomes in our cells, with the exception of gametes, which contain a single set of 23 chromosomes and the complete set of 23 chromosomes is obtained when an egg is fertilized. A person gets half of their nuclear DNA from their father and half from their mother. In this way, the nuclear DNA in humans constantly changes from one generation to the other. This much larger source of DNA is responsible for the physical characteristics of individuals and is unique to each person, with the exception of identical twins. Nuclear DNA is unique to each individual and its profile points toward individual identity. As more is learned regarding the frequencies of various sequences of DNA bases, an improved likelihood of finding a particular sequence is determined. As dozens to hundreds of locations on nuclear DNA are often tested and their sequence determined, a statistical statement of the odds of someone else who is not the suspect or missing person presenting the same DNA profile can be created.

The Master Molecule in Witness Box (with Some Case Studies)

The advent and continual evolution of the science and capabilities of forensic DNA testing directly impact the ability to identify and reassociate individuals who were killed in mass loss of life incidents, as well as the ability to identify individuals in single graves. This ability began in the 1980s with the first application of forensic DNA testing in a courtroom setting. At that time, forensic DNA testing was a relatively new science and many of its principles and intricacies were not well understood. As it was a new science that was not well understood, it was required to undergo and successfully navigate a series of in-depth validation studies. The series of validation studies that forensic DNA testing was required to undergo prior to widespread acceptance created a standardized system that was acceptable around the world and that was far more objective in nature than many other forensic sciences. As the odds of a DNA match between a recovered and tested sample and some type of reference sample are dependent upon the frequency of alleles, bases, or markers, and as the frequency of these alleles occurring within a population is known from previous testing, a statistical probability of a match can be determined. Being able to assign such statistical modeling combined with the testing being objective and reproducible are several of the key reasons forensic DNA testing has often been called the "Gold Standard" of forensic testing [4].

DNA testing supports the rule of law by not only matching unknown samples to known individuals, but also by exonerating those who are charged or suspected of crimes and in which the DNA testing reveals it is not their sample left at a crime scene. In this way, DNA testing can remove suspects from suspicion, as well as link samples from crime scenes to individuals as well as to samples from other crimes scenes, thus aiding in the understanding of who may have committed criminal activities as well as the relatedness of various crimes. The more robust a sample collection and DNA

testing system is, often times the greater its impact on society by solving crimes, removing criminals from the streets, and lowering overall crime rates. In addition, testing of samples from past cases helps to exonerate those who may have been incorrectly convicted of crimes they did not commit and this has led to several Innocence Projects in which cases are reviewed to determine the availability of a biological sample and the circumstances surrounding the conviction and if doubts about the conviction are created in the reviewers. The testing of samples in this manner has led to hundreds of people being exonerated from past convictions. By reducing crimes rates, which results in fewer victims, and releasing suspects from incorrect suspicion of committing crimes, the state is held more accountable to the people, thus supporting human rights.

One example of how a robust DNA testing project can reduce burglary was performed by the FBI in Denver, Colorado. The focus of this project was to collect and quickly test biological samples that were collected from crime scenes related to burglary. Samples collected were compared to various suspect DNA databases as well as to DNA profiles obtained from other burglary crime scenes collected in Denver. The results of this project demonstrated how linking DNA profiles obtained from crime scene samples across a city can develop a better understanding of the individuals involved in such crimes and aid in investigations. Once one suspect is taken into custody and their DNA profile linked to a series of crimes, knowing with whom they associate may lead to additional arrests. In the case of the Denver project, the crime rate for burglary fell significantly and the consequential decrease cost to the police, courts, and property owners more than outweighed the cost of the DNA testing program, meaning the implementation of a robust DNA testing system to combat burglary resulted in a net financial savings to the city, as well as an incalculable mental benefit to homeowners who did not become victims of burglary [5].

As our understanding of the power of DNA testing has increased and improved, how it can be better utilized in legislation has changed. A DNA profile from a crime scene remains the same with the passage of time, unlike the memory of a victim, which may be inaccurate and change with time. Strong emotions may also suppress memories, or alter them to the point that would lead to false convictions. Various legislative changes incorporated the unchanging nature of DNA to develop relatively unique strategies to more fully gain the benefits of forensic DNA testing. For example, the testing of rape kits can often provide a DNA profile from the rapist, even if the rape occurred decades ago. Some states permit the charging of a DNA profile obtained from a crime scene with criminal counts. This effectively froze time passing in the statute of limitations and the person can later be arrested and charged with the crime once their profile is obtained [6]. Another lesson learned is the value of creating local DNA database, in addition to a national database. Most crimes are committed by people who live in, or nearby, the city where the crime occurred, thus providing greater value to a local database. Also, local authorities can more often than not prioritize cases and obtain results in a shorter amount of time, which results in expediting justice.

DNA Forensic Investigation

Of all the functions of the police, investigation is the most important and vital one. In the constantly evolving socio-economic scenario, the criminals using sophisticated tools and techniques commit more and more crimes. In order to overcome these complexities, the police all over the world are depending more and more on scientific methods of investigation. A wide range of scientific techniques is now available for analysis of varied nature of objects and materials encountered in the process of Commission of crime by the culprit in and around the crime scene, on the suspect and victim. The study of such material evidence also known as objective evidence or physical evidence applying the latest scientific tools and techniques for proving the guilt or innocence of the accused by the courts of law is broadly known as forensic science.

The application or aid of science to the crime investigation is fundamentally one of reconstruction, that is, trying to assist in determining what happened, where it happened, when it happened, and who was involved. It is not concerned with, and cannot determine, why something happened. Forensic analysis is performed on evidence to assist the police and the court in establishing physical facts so that criminal or civil disputes can be resolved. It is the job of the forensic scientist to translate the legal inquiry into appropriate scientific questions and to advise the investigating officer and or the judiciary on the capabilities, limitations, and results of the analytical techniques.

In forensic science, the laws of various facets of science are applied in conducting an analysis to determine the nature and characteristics of physical evidence collected in the process of crime or civil investigation. Using the scientific methods, inferences are drawn about how the evidence can build into the crime and criminal. These inferences are thus connected to the events that may or may not have taken place in connection with said evidence. The law defines elements of a crime; science contributes information to assist in determining whether an element is present or absent.

It is an established fact that the criminals while committing crimes either due to carelessness or due to anxiety, or due to contact with other objects leave traces at the scenes and these are the basis for scientifically exploiting their culpability. This physical evidence when located, collected, preserved, and forwarded for the scientific evaluation, the report is bound to have in rumors potential in linking the criminal to the crime scene, victim, or any other circumstances leading to the crime. Moreover, evidence collected and based on scientific evolution and report is unbiased, universally acceptable, and also stands the test of time.

There is a time limit for sending physical evidence to forensic laboratories for scientific analysis. The investigating officer should collect and forward all types of physical evidence along with controlled samples so as to reach the forensic laboratories as soon as possible from the date of crime. Physical evidence sent after around 30 days should not be accepted except in rare cases that too with a proper justification.

The success of the scientific effort in arriving at the truth depends both on the role of the investigating officer and the forensic scientists that are in the thorough search and collection of evidence in the proper way by the investigating officer and then careful analysis through the right tools and techniques by the scientists.

Purpose of physical evidence in crime investigation: The world physical evidence denotes all evidence having certain physical dimensions such as size, save, pattern commands length width height, volume, etc. which can be preserved, lifted, collected, packed, and forwarded for the scientific analysis to the space list concern. Physical evidence can alternatively be called as material evidence, objective evidence, circumstantial evidence, or indirect evidence in different contexts. Physical evidence includes all naturally occurring substances as well as machine or man-made objects in the universe.

Goal of Physical Evidence: The goal of physical evidence examination is to provide useful information for the criminal investigators in solving crimes and for courts of law during the education of these cases. The following leads can be obtained from the examination of physical evidence in the process of investigation.

The corpus Delicti: The corpus delicti refers to those essential facts which show that a crime has taken place. For example com tool marks, broken doors or windows, ransacked rooms, and missing valuables would be important in establishing the burglary has taken place. Similarly, in an assault case, the victim's blood or torn clothing could be important pieces of physical evidence.

Linkage: The linkage is one of the most common and an important aspect that physical evidence can help to establish. Blood, hairs, clothing fibers, cosmetics, and other items from the victim may be transferred to a perpetrator. Items found in a suspect's position can sometimes be linked to the victim, for example through the comparison of bullets with a weapon seized from the suspect the suspect can be linked with crime. It is also possible that evidence is transferred from a perpetrator to the victim in rape, murder, and assault cases.

Linking a person to a crime scene: It is one of the most vital steps in a crime investigation. Numerous types of evidence may be deposited by the person committing a crime, including fingerprints, footprints, blood, hair, fibers, and soil. In addition, the type of weapons or objects used may also leave evidence for example bullets and cartridge cases or tool marks. Depending on the type of crime, various kinds of evidence from the scene may be carried away intentionally as a story property and unintentionally as a transfer of tracks evidence such as carpet fibers or hairs on the criminal's shoes or clothing. These materials are extremely useful in linking an individual to a particular crime scene.

Disproving or supporting a witness's testimony: Physical evidence analysis can often indicate conclusively whether a person's version of a set of events is credible, or whether an alley put forth is convincing or not. For example, the examination of a car that fits the description of a hit-and-run vehicle might reveal blood on the underside of the bumper. If the owner of the vehicle claims he hit a dog, laboratory tests on the blood can reveal whether the blood is from a dog or from a human and further testing for DNA profiling may reveal the truth relating to the accident and victim.

Identification of a suspect: The best evidence for identifying the suspect is his or her palm prints and evidence such as blood, semen, saliva, skin tissues, etc. collected for DNA fingerprinting examination. A fingerprint found at the scene, and later identified as belonging to a particular person results in an unequivocal identification of that person has having been at the scene. The term identification when applied to people really means individualization.

Providing investigative leads: Physical evidence analysis can assist the investigator in pursuing a productive path, by providing close from the characteristics of the physical evidence. In a hit-and-run case, for example, examination of a chip of paint found in victim's clothing could be used to provide information on the color and possibly the model and year, of the automobile involved. With the rapid emergence of computers add fingerprint and DNA databases, the ability of physical evidence to provide investigation leads has increased enormously.

Crime scene reconstruction: Transient evidence such as odor, temperature, imprints, marks stains-pattern evidence like bloodstain patterns, skid mark patterns, guns out at residual patterns, projective trajectory patterns, glass fracture patterns, etc. and conditional evidence such as light, smoke, fire, what are, piled up newspapers, etc. will play a key role in reconstruction of the events surrounding the crime scene and the nature of office. Sometimes it may also give valuable leads regarding the perpetrator based on the individual traits in relation to the evidentiary pattern.

Developing DNA Databases and Revolutionizing the Forensic Investigation

The basic premise of using DNA profiles to combat crime and/or identify the missing from unknown samples is based upon putting the DNA profiles obtained from samples at crime scenes into one database and comparing to DNA profiles obtained from suspects that are stored in another database, or from DNA profiles obtained from skeletal remains and comparing them to DNA profiles of family members who are missing family members, or personal articles of the missing that might contain some of their DNA. The ability of DNA testing to match profiles obtained from unknown samples to a known reference grows as the size of the DNA databases grows. For criminal investigations, from whom samples can be collected for entering into the "known" or reference database, is a primary determinate on creating a successful DNA matching project. When databases were first being created, most legislation required samples only be collected from convicted felons and even then, for only the most serious crimes such as murder, rape, and crimes against children. As many of these felons were in prison for an extended period, this meant that testing of samples from crime scenes had a low chance of resulting in a match. As the power of DNA database became better understood, legislation changed to collect samples from a wider population [7]. The types of crimes from which suspects/convicted would be collected grew and with each increase in collection categories, the rate

of matching crime scene samples against known samples increased. Some nations and states included collecting samples for lesser crimes, such as traffic offenses, which dramatically increased the power of DNA database. Some nations and states in the United States passed all arrestee legislation, in which samples were collected from individuals who were arrested of various crimes. If the individual was later exonerated and/or the case dismissed, their samples were to be expunged from the database of known samples, though in some cases this could be a lengthy process. While some nations are considering total population DNA database where everyone is required to provide a sample, such efforts have been strongly resisted based upon concerns for civil rights and liberties.

As stated above, the power of a DNA database to link DNA profiles obtained from unknown samples to know samples is directly related to the number of samples contained within the database. The UK undertook an aggressive collection effort in which samples were collected from individuals involved in low-level crimes, as well as those convicted of serious crimes. This encompassing database resulted in the UK system having a match rate of an unknown sample to a known person of approximately 60% [8]. In the context of Bosnia and missing persons due to conflict from the breakup of the former Yugoslavia, as the database of family members who are missing family members grew, the chance of a set of unknown skeletal remains matching family references exceeded 80%. The number, and from whom, samples are collected is a prime factor that will determine the power of a DNA database.

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